Phenotyping asthma attacks: clinical, physiological and inflammatory characteristics of acute asthma

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Thesis submitted to the University of Nottingham for the degree of Doctor of Philosophy

May 2025

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Abstract

Background

Asthma is increasingly recognised as a heterogeneous disease, encompassing a range of phenotypes and endotypes. This evolving understanding of the pathophysiology of stable asthma has enabled the development of targeted anti-inflammatory treatments that have significantly improved clinical outcomes. Unfortunately, this is not the case with asthma attacks (AA), as they remain poorly understood and largely treated the same way since the 1950s. This is mainly owed to the lack of longitudinal investigations of asthma at the time of acute symptoms worsening and during periods of recovery, and the subsequent poor understanding of the pathophysiology of AAs. This is further confounded by the absence of a clear definition of these episodes, and the lack of easily performed lung function assessments and reliable attack-specific inflammatory biomarkers, which, collectively, has fostered a one-size-fits-all approach to managing these episodes irrespective of the underlying pathophysiological mechanism.

Although the underlying pathophysiology of asthma is broadly similar between children and adults, it is widely acknowledged that distinguishing between asthma and non-asthma-related wheezing in children can be challenging[1]. In addition, ethical considerations and practical difficulties associated with conducting research in paediatric populations further influenced the study design. Consequently, the study team elected to focus exclusively on asthma attacks in adults. The literature cited in the background chapter predominantly relates to adult patients.

Aim

This thesis aims to investigate asthma attacks, with a specific focus on objectively assessed airflow obstruction and the underlying inflammatory phenotypes that characterise these events. Additionally, it will explore the utility of point-of-care biomarkers in phenotyping inflammation during AAs and their potential role in guiding and predicting treatment responses.

Results

Chapter 2: Audit of asthma attack admissions

A significant proportion of patients hospitalised with acute asthma do not have documented objective evidence of airflow obstruction. Notably, a sub-group of patients repeatedly admitted for AAs is responsible for a significant number of AA admissions annually. These patients are more likely to have comorbidities, asthma-mimicking conditions, and repeated admissions during which no evidence of airflow limitation is recorded.

Chapter 3: APEX3- Cohort overview and clinical characteristics

Confirming findings from the Audit, at least a fifth of patients hospitalised with AAs do not have objective evidence of acute asthma deterioration. The use of point-of-care assessments for airflow obstruction and inflammatory profiling during AAs was well tolerated by the majority of patients. Furthermore, fractional exhaled nitric oxide (FeNO) measurement during acute episodes may have clinical utility in identifying inflammatory phenotypes, which, as demonstrated in the present study, appear to be heterogeneous, similar to those observed in stable asthma.

A significant proportion of AA admissions are accompanied by small airways impairment, either with impairment of the large airways or in isolation as in the small airway disease asthma phenotype. Importantly, discordance between airflow limitation measured by spirometry and impulse oscillometry has been observed, likely owed to the involvement of small airway dysfunction not detected using conventional lung function measures.

Chapter 4: APEX3- Comparison between Likely Acute Asthma-Related Admissions (LAARA, n= 64) and Likely Not Acute-Asthma Admissions (LINAA, n= 19)

Comparison between LAARA and LINAA demonstrated no significant differences. However, findings from the comparison between LAARA and NARA (Non-Asthma-Related Admissions) (Appendix G) were largely consistent with those of the Audit. The NARA group are more likely to be younger with increased incidence of asthma comorbidities and mimics and high healthcare utilisation for asthma including frequent

hospital admissions. Interestingly, all the patients without evidence of acute airflow limitation had a prior diagnosis of asthma, which may have influenced the interpretation of their symptom worsening. Patients with evidence of acute airflow limitation had significantly higher nasal IL-13 levels, raising the potential for IL-13 as a marker of acute T2-related worsening of asthma symptoms but this requires further investigation.

Chapter 5: APEX3- Analysis of follow-up visits

Longitudinal analysis from this study demonstrated that a significant proportion of patients had lung function that remained largely unchanged at recovery compared to during hospitalisation. In those whose lung function improved, the small airways, as assessed using both spirometry and forced oscillation technique (FOT), showed significant recovery, suggesting potential involvement of the small airways in the pathophysiology of acute asthma.

The response to treatment with oral corticosteroids (OCS) varied depending on the underlying inflammatory phenotype, with those with a T2-high attack phenotype demonstrating greater lung function improvement. However, symptom improvement did not significantly differ between phenotypes, highlighting the limitations of traditional symptom-based treatment algorithms. Importantly, point-of-care FeNO measurement demonstrated high specificity for identifying patients likely to respond to treatment, suggesting this biomarker may have utility in a future precision medicine approach to managing acute asthma episodes.

Conclusion

This thesis provided an overall insight into asthma attacks and highlighted the potential limitations of commonly used guidelines for assessing and treatment acute episodes of asthma with little emphasis on objective confirmation of airflow limitation. The findings also highlighted the potential utility of precision medicine approaches, currently utilised for the management of the stable asthma, in guiding assessment and treatment decisions for acute asthma and improving upon the current suboptimal one-size-fits-all approach.

Thesis outline

This thesis will be divided into 6 chapters as follows

Chapter 1: Background and introduction

This chapter provides an overview of stable asthma, followed by a more detailed review of asthma attacks literature with an emphasis on definition and differences between asthma control and attacks, epidemiology and disease burden, pathophysiology and aetiology, risk factors, asthma mimics, and the treatable traits.

Chapter 2: Audit of asthma attack admissions

This chapter presents a retrospective audit of medical records from adult patients admitted to a secondary respiratory centre with suspected asthma attacks over a one-year period prior to the COVID-19 pandemic. The primary focus is on the documentation of objective evidence supporting the diagnosis of an asthma attack. This chapter discusses characteristics of patients admitted without documented objective evidence of airflow obstruction and those with repeat admissions, highlighting the overlap between these groups and previous description of patients with asthma-mimicking conditions.

Chapter 3: APEX3- Cohort overview and clinical characteristics

This chapter provides an overview of the cohort recruited for the prospective clinical study of asthma attack admissions at Nottingham City Hospital: Asthma Phenotyping Exacerbations 3 (APEX3). It describes and discusses the demographic and clinical characteristics of the patients identified, along with evidence of airflow limitation suggestive of acute asthma. In addition, the chapter discusses the utility of point-of-care assessment of lung function and inflammation, the heterogeneity of AAs, the potential involvement of the small airways, and the discordance observed between lung function measures, spirometry and FOT.

Chapter 4: APEX3- Comparison between Likely Acute Asthma-Related Admissions (LAARA, n= 64) and Likely Not Acute-Asthma Admissions (LINAA, n= 19)

This chapter describes and compares the demographic features, clinical characteristics, and admission details of patients with and without prospectively assessed, objectively defined evidence of airflow limitation suggestive of acute asthma from the APEX 3 study.

Chapter 5: APEX3- Analysis of follow-up visits

This chapter describes the clinical characteristics and biological response following hospital admission and treatment with OCS for AAs. Between visit changes in lung function and symptoms burden are discussed. Changes in lung function and symptom scores at Visit 2 compared to Visit 1 in the T2-high and T2-low asthma attacks and the utility of inflammatory biomarkers profiling in predicting treatment response are highlighted and discussed.

Chapter 6: Summary of findings, discussion, and suggestions for future research

This chapter provides a general overview of the findings presented throughout this thesis, summarises the main outcomes and their implications for clinical practice, and concludes with suggestions for future research to address current knowledge gaps and guide the development of more precision medicine approaches for the assessment and treatment of acute asthma.

Declaration of work personally performed

Although the primary intent of my PhD was to conduct clinical research on asthma attacks, I started my studies during the national COVID-19 lockdown, during which non-covid related clinical research was temporarily suspended. Consequently, it was sensible to conduct a retrospective, non-clinical pilot study on asthma attack admissions, which significantly informed the design and implementation of the subsequent clinical phases of my PhD.

Approvals for the Audit were submitted by my supervisor, Dr Matthew Martin, while I awaited the completion of my contracts and administrative processing. My supervisors and I cooperatively designed the audit, and continuous refinement of the study methodology throughout the audit phase were conducted by myself with support from my supervisors, Professor Tim Harrison and Dr Matthew Martin. All data collection, from both digital clinical records and handwritten scanned notes of asthma attack admissions (N= 311), was undertaken solely by me, and I was responsible for the full data entry process into the study database.

The protocol, study-specific documents, and ethical approval documents for APEX3 were all designed, prepared, and submitted to the regulatory bodies by myself. I conducted and managed the study entirely, including the screening, recruitment, and consent of all participants (N= 100). I conducted all clinical assessments during both hospitalisation and follow-up visits, including sputum induction. Data entry for the study was completed solely by myself.

Cytokine bioplex assays and sputum samples were processed by the Nottingham Respiratory Research Unit scientists. Nasal brushing samples for RNA and DNA extraction were processed by PhD fellows at the respiratory molecular genetics group at the University of Nottingham. Blood samples were processed at local laboratories by hospital laboratory staff members.

I conducted all the data analysis and interpretation of the results myself with support from my supervisors, Dr Matthew Martin, Professor Tricia McKeever, and Professor Tim Harrison. The entire thesis was written by myself, with review and feedback provided by my supervisors.

Acknowledgements

First and foremost, I would like to thank Allah (My God) for giving me the strength and patience to complete this work.

The completion of this PhD wouldn't have been possible without the valuable support of many people.

I would first like to start by expressing my sincere gratefulness to Dr Matthew Martin for his invaluable support throughout my PhD journey. His commitment and guidance have greatly enhanced the quality of my work and have made this experience much more manageable than it otherwise could have been.

I would also like to express my sincere gratitude to Professor Tim Harrison for allowing me this opportunity to engage in clinical research, especially at times when this was not realistically possible. His valuable guidance and expertise have significantly strengthened the quality of this PhD.

I would like to express my deep gratitude to Professor Tricia McKeever, who joined the supervisory team later in my PhD journey; her academic guidance and statistical expertise have been invaluable and greatly appreciated.

I would also like to thank the Nottingham Respiratory Research Unit team, who helped me get hands-on exposure and experience with clinical research when I first joined the team. This support has helped me conduct clinical research much more effectively.

To my wife, Dareen, thank you for your support during the most difficult times, when your support was needed the most; this wouldn't have been accomplished without you being by my side. To my children, Amal, Omar, and Hamza I know I have been busy, and I have missed many moments. I have wanted to spend as much time with you as you want to spend time with me, but this work has carried me away a bit. However, this work is nearly finished, and I will soon be back to full parenting duties.

To my parents, your prayers have strengthened me and are the reason behind all my achievements. May God bless you.

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List of abbreviations

AA	Asthma attacks
AAQLQ	Acute Asthma Quality of Life Questionnaire
ABRA	Treating eosinophilic exacerbations of asthma and COPD with benralizumab
ACQ	Asthma Control Questionnaire
AHR	Airway hyperresponsiveness
APEX3	Asthma Phenotyping Exacerbations 3
ASM	Airway smooth muscle
ATS	American Thoracic Society
AUC	Area under the curve
AWI	Airway inflammation
AWR	Airway remodelling
BDR	Bronchodilator response
BEC	Blood eosinophil count
BMI	Body mass index
BNC	Blood neutrophils count
BPD	Breathing Pattern Disorder
BTS	British Thoracic Society
CompEx	Composite endpoint for Exacerbations
COPD	Chronic Obstructive Pulmonary Disease
CRP	C-reactive protein
CT	Computed tomography
CXR	Chest radiograph
EAO	Evidence of airflow obstruction
ECM	Extracellular matrix
EDAC	Excessive Dynamic Airway Collapse
EMTU	Epithelial Mesenchymal Trophic Unit
FACET	Formoterol and Corticosteroids Establishing Therapy
FE	Frequent exacerbators
FEF	Forced expiratory flow

FeNO	Fractional exhaled nitric oxide
FEV_1	Forced expiratory volume in 1 second
FOT	Forced oscillation technique
FTA	Frequent-time attendees
FVC	Forced vital capacity
GERD	Gastroesophageal Reflux Disease
GERDQ	The gastro-esophageal reflux disease Questionnaire
GINA	Global Initiative for Asthma
GLI	Global Lung Function Initiative
GP	General Practitioner
HADS	The Hospital Anxiety and Depression Scale
HBMI	High body mass index
HRCT	High-resolution computed tomography
HVS	Hyperventilation syndrome
ICD-10	International Classification of Disease 10
ICS	Inhaled corticosteroids
ICU	Intensive care unit
IFN	Interferon
IgE	Immunoglobulin E
IL	Interleukin
ILC1	Innate lymphoid cell group 1
ILC2s	Innate lymphoid cell group 2
ILO	Inducible laryngeal obstruction
IOS	Impulse Oscillometry
IQR	Interquartile Range
ITU	Intensive treatment unit
LAARA	Likely Acute Asthma-Related Admissions
LBMI	Low body mass index
LFE	Less frequent exacerbators
LINAA	Likely Not Acute-Asthma Admissions
LLN	Lower limit of normal
LOCA	Likely Out of Control Asthma Admissions

LOS	length of hospital stay
LRTI	Lower Respiratory tract infection
MARS	The medication adherence report scale
MARS-5	The medication adherence report scale-5
MCID	Minimal clinically important difference
MCT	Methacholine challenge test
MPR	Medication possession ratio
MTA	Multiple-time attendees
NACAP	National Asthma and Chronic Obstructive Pulmonary Disease Audit Programme
NARA	Non-Asthma-Related Admissions
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NOO	No Obvious Obstruction
NPV	Negative predictive value
NRAD	National Review of Asthma Deaths
NUH	Nottingham University Hospital
OCS	Oral corticosteroids
OR	Odds ratio
OSA	Obstructive sleep apnoea
PaCO2	Partial pressure of carbon dioxide in arterial blood
PaO2	Partial pressure of oxygen in arterial blood
PC20	Provocative concentration causing a 20% fall in Forced Expiratory Volume in 1 second (FEV_1)
PCR	Polymerase chain reaction
PD20	Provocative dose of methacholine that results in a 20% fall in in Forced Expiratory Volume in 1 second (FEV_1)
PEFR	Peak expiratory flow rate
POC	Point-Of-Care
PPB	Parts per billion
PPV	Positive predictive value

PRISMA	Phenotyping the Responses to Systemic Corticosteroids in the Management of Asthma Attacks
PVCDI	The Pittsburgh vocal cord dysfunction index
R5-R20	Resistance heterogeneity between 5 and 20 Hz (peripheral resistance)
R5	Resistance at 5 Hz (total resistance)
ROC	Receiver-operating characteristic
RV	Rhinovirus
SABA	Short-Acting Beta-Agonists
SAD	Small airway dysfunction
SARP	Severe Asthma Research Program
SCS	Systemic corticosteroids
SD	Standard deviation
SEC	Sputum eosinophil count
SIGN	Scottish Intercollegiate Guidelines Network
SNC	Sputum neutrophil count
SNOT-22	Sino-nasal Outcome Test
SpO2	Peripheral oxygen saturation
STA	Single-Time Attendees
TLC	Total lung capacity
TNF	Tumour necrosis factor
TSLP	Thymic stromal lymphopoietin
TT	The treatable traits
URTI	Upper respiratory tract infection
VCD	Vocal cord dysfunction
WHO	World Health Organization

Publications arising from this thesis

Thematic poster, The ERS Congress 2024, Vienna. **M Aljehani**, T Harrison, M Martin, Measures of lung function and inflammation in patients hospitalised with severe asthma attacks and 2 weeks later, Vienna, 2024 European Respiratory Journal 2024 64 (suppl 68): PA4765; DOI: https://doi.org/10.1183/13993003.congress-2024.PA4765

Thematic poster, The ERS Congress 2024, Vienna. Paula Cristina Andrade Almeida, **M Aljehani**, T Harrison, M Martin, The assessment of tidal expiratory flow limitation and small airways disease using forced oscillation technique during hospitalization for asthma attacks and recovery, Vienna, 2024, European Respiratory Journal 2024 64(suppl 68): PA3463; DOI: https://doi.org/10.1183/13993003.congress-2024.PA3463

Thematic poster, The ERS Congress 2023, Milan. **M Aljehani**, T Harrison, M Martin, Phenotyping severe asthma attacks in patients hospitalised with acute asthma, Milan, 2023, European Respiratory Journal 2023 62 (suppl 67): PA4742; DOI: https://doi.org/10.1183/13993003.congress-2023.PA4742

Oral presentation, The ERS Congress 2023, Milan. Paula Cristina Andrade Almeida, **M Aljehani**, T Harrison, M Martin, Comparison of forced oscillation technique (FOT) with spirometry measures in acute asthma, Milan, 2023 European Respiratory Journal 2023 62(suppl 67): OA754; DOI: https://doi.org/10.1183/13993003.congress-2023.OA754

Thematic poster, The ERS Congress 2022, Barcelona. **M Aljehani**, T Harrison, M Martin, Phenotypic features of >300 suspected severe asthma attacks, Barcelona, 2022 European Respiratory Journal 2022 60 (suppl 66): 105; DOI: https://doi.org/10.1183/13993003.congress-2022.105

Chapter 1: Background and introduction

Asthma

Definition

Asthma is a heterogeneous, chronic inflammatory disease of the airways defined by the presence of a history of variable shortness of breath, wheeze, cough and other respiratory-related symptoms, along with reversible airflow limitation[2]. This airflow limitation can become irreversible[3,4] in long-standing[5] and severe asthma[6,7] due to airway inflammation and remodelling[8]. However, the variability of the intensity of symptoms and, most importantly, the variability of the degree of airflow limitation are what distinguish asthma from other obstructive respiratory diseases.

Epidemiology and disease burden

Asthma is a prevalent disease affecting more than 300 million people globally[9]. There has been an evident increase in asthma prevalence worldwide since the 1950s[10], which is expected to continue[10,11]. In 2015, 358 million people had asthma, making it the most prevalent chronic respiratory disease globally[12]. In the UK, a nationwide study estimated a prevalence of clinically diagnosed asthma of 15.6%, with 9.8 million people affected[13]. Furthermore, the Asthma + Lung UK estimates that around 160,000 people are diagnosed with asthma every year, making asthma the commonest respiratory disease in the UK[14].

Asthma is associated with a high burden of direct and indirect costs, and it is estimated that asthma costs the UK at least 1.1 billion every year[13]. Much of the direct cost can be directly attributed to the costs of managing asthma attacks[15]. The indirect costs of asthma can also be significant and are mainly caused by loss of productivity due to foregone labour[16]. Also, asthma is a major cause of poor quality of life and hospital admissions. Globally, asthma accounts for 10 million years of life lost, 16 million years lived with disability, and 26 million disability-adjusted life years[12].

Asthma diagnosis

The diagnosis of asthma is based on a structured clinical assessment guided by the presence of a history of respiratory symptoms indicative of airflow obstruction, such as

shortness of breath, coughing, wheezing, and chest tightness, along with objective evidence of expiratory airflow obstruction variability[17]. Symptoms of asthma may improve with treatment or spontaneously, and it can be difficult to diagnose asthma after starting treatment. Thus, the Global Initiative for Asthma (GINA) recommends testing and documenting evidence for asthma before starting treatment[17]. There are a number of diseases that can mimic asthma symptoms. These include inducible laryngeal obstruction, bronchiectasis, and dysfunctional breathing; therefore, the pattern of asthma symptoms is important, and it is recommended the variability of airflow limitation is confirmed by more than one objective test.

Symptoms of asthma include intermittent and variably intense shortness of breath, coughing, wheezing, and chest tightness that worsen at night and are associated with well-defined triggers, including exercise, allergens, and viral infections. Airflow variability in asthma can be confirmed using a number of objective tests, including positive bronchodilator responsiveness for variability using spirometry or peak exploratory flow, excessive daily peak flow variability, improved lung function after treatment, positive bronchial challenge test, or increased variability of lung function measures between clinical visits[17].

A number of objective tests that help support the diagnosis of asthma are available. These include skin prick tests, allergen-specific IgE levels for atopy status, fractional exhaled nitric oxide (FeNO), and blood eosinophil count (BEC); however, these measures only increase the likelihood of asthma and do not rule out the diagnosis if negative[17]. Therefore, the new joint British Thoracic Society (BTS), National Institute for Health and Care Excellence (NICE), and the Scottish Intercollegiate Guidelines Network (SIGN) (BTS/SIGN/NICE) diagnostic pathway recommends that an asthma diagnosis should be based on a clinical history suggestive of asthma supported by evidence from objective tests [18].

Pathophysiology, aetiology, and heterogeneity of stable asthma

Pathophysiology

The pathophysiology of asthma is complex and involves multiple biological interactions between gene products, environmental factors, and comorbidities. Asthma

pathophysiology can be categorised into three deeply interrelated areas: airway inflammation (AWI), airway hyperresponsiveness (AHR), and airway remodelling (AWR). The small airways have also been suggested to play a key role in the pathophysiology of asthma, but this is not well understood.

Airway inflammation

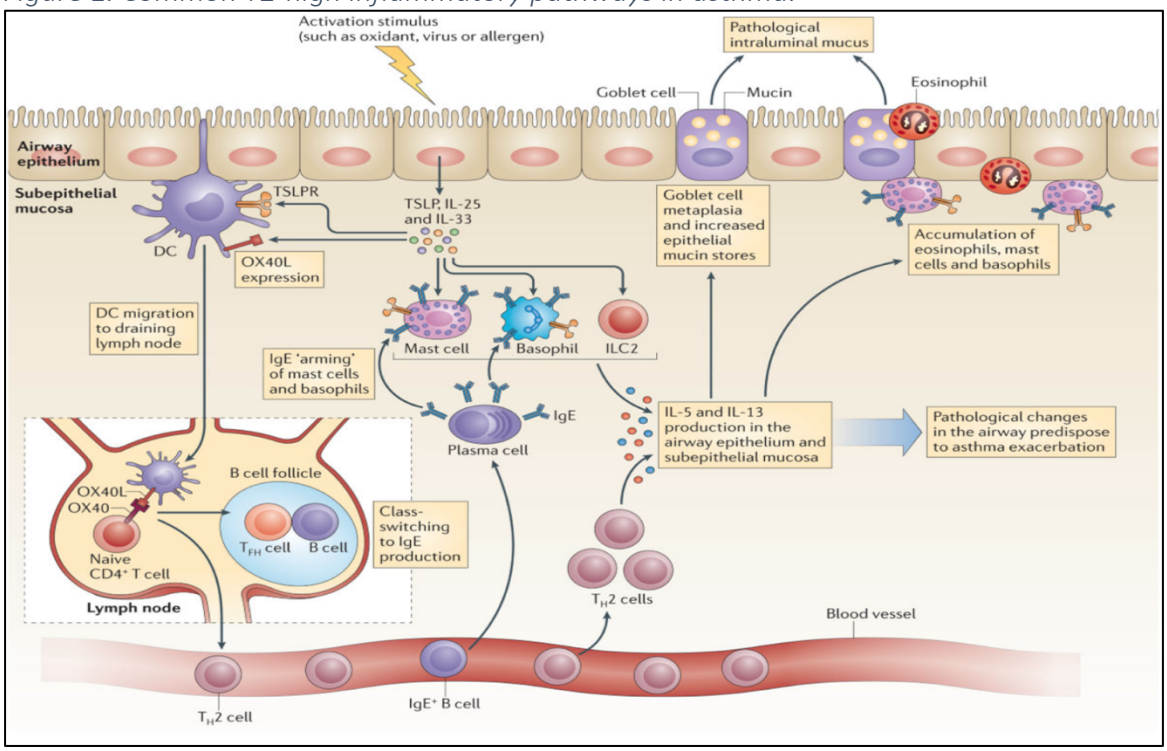
Central to the pathophysiology and manifestations of asthma is the inflammation of the lower airway[19,20]. AWI in asthma contributes to airway hyperresponsiveness, mucus hypersecretion, and remodelling of the airways and is a consistent feature among different phenotypes of asthma independent of disease severity[20-22]. AWI involves complex interactions of cells and mediators within the airways, resulting in pathophysiological characteristics and disease manifestations such as shortness of breath and wheezing.

Understanding of the immune response of $CD4^+$ T-cells and their subtype cells (T1 and T2) has led to the realisation of the role of T2 cells and their cytokines in driving eosinophilic airway inflammation[23]. Subsequently, a landmark study by Wenzel et al. led to the identification of the well-established, widely adapted asthma endotype classification based on the presence of airway eosinophilia (T2-high and T2-low)[24].

The innate immune response originates at the airway epithelial barrier[25], and an impaired epithelial barrier is a common feature in asthma patients[26,27]. T2 subtype cells stimulate T2-high response by secreting IL-4, IL-5, IL-9, and IL-13[28]; IL-4 and IL-13 are responsible for B cell activation and subsequent IgE production, IL-5 promotes bone marrow eosinophil production, and IL-9 activates mast cells[28]. IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) are epithelial-derived alarmins that initiate T2-high pathways in response to infections and allergen exposure[25,29]. They are found at increased levels in asthma patients compared to healthy individuals, correlate with disease severity[30-33], and are related to remodelling of the airways in severe asthma[34]. Alarmins trigger innate lymphoid cell group 2 (ILC2s) activation, which produces IL-5 and IL-13, promoting and facilitating T2-high response[35]. The ILC2 pathway has a key role in augmenting T2-high responses due to their increased ability to produce IL-5 and IL-13 compared to T2 cells[36] (Figure 1). Activated dendritic cells, often due to an external insult, can also promote alarmin production and subsequent T2-high

inflammation[25,29]. T2 cytokines stimulate T2 responses, resulting in high expression of IgE, ILC2s, eosinophils, basophils, mast cells, and T2 cells[37].

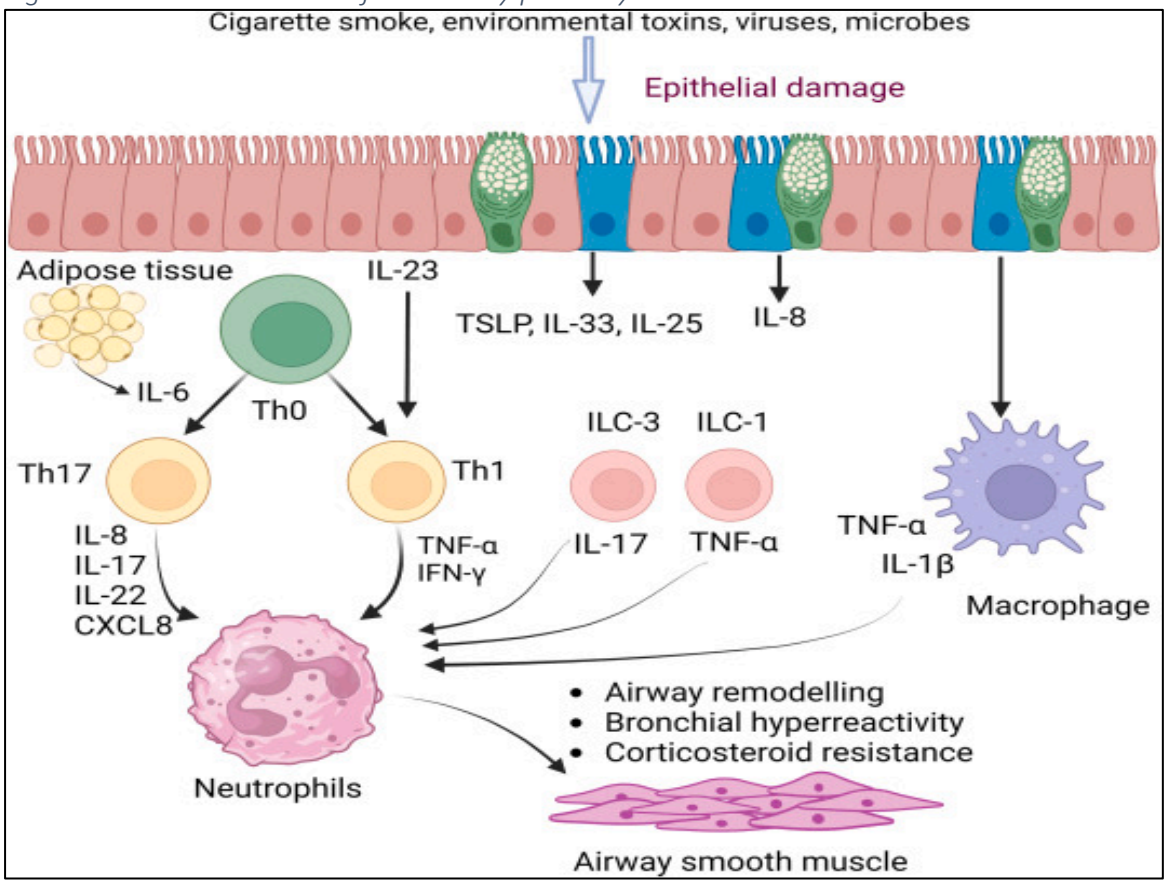
Figure 1. Common T2-high inflammatory pathways in asthma.



Reproduced from Fahy et al.[23]

T2-low asthma, on the other hand, is characterised by the absence of T2-high biomarkers, with or without neutrophilia[38,39], and is mediated by T1/T17 pathways (Figure 2). The T1 pathway is stimulated through IL-2 and interferon-γ (IFNγ)[28]. IFNγ is increased in almost half of the patients with severe asthma and is associated with amplified inflammatory infiltrates, increased airway resistance and AHR, and steroid refractoriness[40]. The T17 pathway is mainly induced by the production of IL-17A and IL-17F[41] and regulated by IL-23[42]; T17 is characterised by poor steroid responsiveness[41]. Both IL-17A and IL-17F are commonly elevated in asthma patients with different severities of disease compared to healthy individuals[43]. In addition, IL-17 and IL-22 promote smooth muscle cell proliferation and collagen deposition[41]; IL-17F has been linked to the asthma "exacerbation-prone" phenotype[44]. T17 activity is important in the activation and maintenance of neutrophils in the airways of asthma patients[41].

Figure 2. Common T2-low inflammatory pathways in asthma.



Reproduced from Adrish and Akuthota[45].

It has been suggested that corticosteroids may facilitate persistent T2-low inflammation through increased T1 cell recruitment[46]. Similarly, evidence also suggests that inhibition of T2-high pathway through corticosteroids promotes IL-17 secretion and facilitates T2-low inflammation[47]. T2-low asthma is poorly understood, and it is not clear how airway neutrophils are recruited and maintained. It has been argued, however, that the presence of airway neutrophilia could be due to the effect high dose ICS have on neutrophil apoptosis[48] and that the lack of T2-high biomarkers could be due to steroid therapy[29].

Airway hyperresponsiveness

Airway hyperresponsiveness is defined as the increased reactivity of the airway smooth muscle in response to external stimuli, resulting in airway constriction and airflow obstruction[49]. The airways of asthma patients have increased reactivity to external triggers, including cold air, allergens, viruses, exercise, histamine, and methacholine[50].

Assessing the level of reactivity using the methacholine challenge test is one of the criteria for diagnosing asthma[17]. Although improvement in airway inflammation has been linked to a reduction in airway hyperresponsiveness[51-54], results in this area are conflicting, and other studies found no association between AWI and AHR[55,56]; the precise role of AWI in the dysfunction of the airway smooth muscle (ASM) and the consequent hyperresponsiveness is not entirely understood[57]. Airway smooth muscle cells play a key role in the contractility and dilation of the airways. Asthma patients have increased mast cell infiltration into the ASM, which has been shown to be associated with the hyperresponsive airways of asthma patients[58]. Contractility and consequent hyperresponsiveness are induced by ASM mast cells release of mediators known for their potent bronchoconstrictive proprieties, including prostaglandin D2, histamine, cysteinyl leukotrienes, and tryptase[59]. Epithelial-derived IL-33 is also important for the maintenance of airway hyperresponsiveness[60], and high mobility box-1 protein augments the contractility of ASM through the induction of Toll-like receptor-4[61].

Airway remodelling

Airway remodelling in asthma refers to the altered pathological structure of the airway, including ASM hyperplasia and hypertrophy, subepithelial fibrosis, goblet gland hyperplasia, and mucus hypersecretion; such changes lead to airway wall thickening, airway lumen narrowing, and airflow limitation[62]. The airway mass of asthmatics is up to 100% and 300% greater in non-fatal and fatal asthma, respectively, compared to healthy individuals[63]. Airway remodelling in asthma correlates with disease severity[64], is a common finding in severe asthmatics[65], and is associated with permanent loss of lung function[66].

Airway remodelling is argued to be a consequence of long-standing inflammation and resultant CD4+ T cell and eosinophilic granulocyte infiltration of the airway[67]. This is supported by evidence demonstrating that improvement in AWI using high-dose ICS is accompanied by improvement in submucosal vascularity and basement membrane thickness[68]; however, evidence also suggests that AWR can occur independent of long-term inflammatory insults[69,70], and even children younger than 6 years old can have features of AWR[71,72]. Further, studies suggest that the degree of AWR is mostly influenced by the severity, rather than duration, of the disease[73]. It is thus suggested

that AWR is a pathognomonic feature of asthma[74] rather than a consequence of a long-standing disease.

Mechanical stress, in the absence of additional inflammation, imposed on the lungs as a result of bronchoconstriction has also been shown to provoke epithelial stress and tissue repair that results in remodelled airways[75]. AAs are an important contributing factor to the remodelling of the airways. Deep inspiration, cough, and bronchoconstriction are all factors that increase airway mechanical load[76]. Deep inspiration has been shown to impose 25% mechanical stress on the lungs compared to 4% during tidal breathing[77]. In addition, thickening of the airway wall has also been found in computed tomography and biopsy samples of patients with chronic cough[78,79]. Further, collagen deposition, mucus secretion, and cell proliferation have been observed to be increased following in vivo methacholine-induced bronchoconstriction[75]. Biopsy samples of allergen-induced AAs in mild asthmatics have shown increased markers of airway remodelling that persist even after inflammatory markers have resolved[80,81]. In addition, markers of airway remodelling, including amphiregulin, activin A, and VEGF, have been shown to be increased in rhinovirus-infected epithelial cells[82,83]. Thus, evidently, AWR is a plausible explanation for the permanent loss of lung function in patients with frequent AAs[84].

AWR is associated with airflow limitation and lung function decline. Increased ASM mass and sub-epithelial fibrosis correlate with airflow limitation[85-87], and cellular infiltration of the airways has been linked to impaired lung function[88]. The association between AWR and AHR, however, is less well understood. While studies have demonstrated that increased ASM mass, sub-epithelial fibrosis, and damaged epithelial barrier contribute to the pathogenesis of AHR[89-91], other studies found no relationship[92], or even an inverse correlation[93] between AWR and AHR.

Small airway dysfunction

Asthma has long been seen as a disease predominantly affecting the large airways, where the small airways, those with an internal diameter of <2 mm[94], are thought to have a minimum physiological impact[95] and referred to as the “quiet zone” as their dysfunction cannot be detected using conventional spirometry[96]; however, evidence suggests that Small Airway Dysfunction (SAD) is important in the pathophysiology of

asthma. The incidence of SAD in asthma patients is high, with studies indicating a prevalence from 50% to 90%; of note, even those with mild disease and a normal FEV_1 have been observed to have a degree of small airway dysfunction[97-100].

Small airway dysfunction is closely related to asthma outcomes. Scichilone and colleagues assessed the relationship between FeNO and asthma control and reported that the concentration of alveolar, but not bronchial, FeNO was found to correlate with the level of asthma control assessed using the asthma control test[101]. Further, Takida et al. investigated the influence of central and peripheral airways on asthma outcomes and concluded that resistance of the small airways, assessed using impulse oscillometry, significantly contributed to disease control, dyspnoea, and asthma-related quality of life independent of the degree of resistance at the proximal airways[102]. Exacerbation-wise, SAD has also been shown to play a role[103-105], and targeting the small airways using fine particles inhaled corticosteroids has shown to be more effective in controlling symptoms and reducing the risk of AAs compared to routinely available inhalers[106-108]. Bourdin and colleagues demonstrated that small-airways dysfunction, as measured using a computerised single-breath nitrogen washout test, was increased in frequent exacerbators compared to non-frequent exacerbators despite similar lung function parameters assessed using conventional spirometry between the groups[104]. More recently, in a sub-analysis of cohorts from the SARP study, $FEF_{25-75\%}$ <lower limit of normal was associated with AHR, persistent symptoms, emergency room visits, and previous ICU admissions and intubation for asthma[109]. Further, $FEF_{25-75\%}$, in spite of normal FEV_1, has been demonstrated to be associated with increasing risks of AAs in children[110].

Although the lung periphery accounts for no more than 10% of the total airway resistance (measured using conventional spirometry)[111], their overall surface area is greater than those of the large airways[112], and airway remodelling can also contribute to the dysfunction of the small airways[113]; therefore, they are (the small airways) at least equally involved (compared to large airways) in the pathology as well as the physiology of asthma[114]. Indeed, Hamid et al. investigated the inflammatory process in the large compared to small airways and found a significantly higher number of activated eosinophils in the lung specimens of the small airways[115]. The same group also later found an increased IL-5 mRNA expression in the small airways compared to

the large airways in surgically resected asthmatic lungs[116]. The small airways are increasingly recognised as a major contributor to airway resistance in both asthma and chronic obstructive pulmonary disease[117-121]. Wagner and colleagues[122] directly assessed the pressure in the small airways using wedged fiberoptic bronchoscopy and concluded that peripheral airway resistance was significantly increased in asthma patients compared to controls despite similar lung function assessed using conventional spirometry. Of note, the degree of peripheral airway resistance correlated well with the degree of pre-bronchoscopy PD_{20} FEV_1. SAD is commonly associated with AHR[123,124]. Telenga et al.[125] investigated the relationship between SAD and AHR severity in 94 mild-to-moderate severe asthma patients on inhaled corticosteroids. In those with SAD (n= 34), assessed using spirometry (average expiratory flow rate at 50% of FVC ≤LLN), AHR severity, along with impairment in measures of lung function including FEV_1, FVC, FEV_1/FVC, were significantly higher compared to those without SAD. Small airway obstruction was significantly associated with AHR independent of demographic features, FEV_1, and inhaled corticosteroid dosage. Interestingly, those with evidence of SAD had significantly increased daily inhaled corticosteroid dosage compared to those without.

Aetiology of asthma

The aetiology of asthma is believed to be multifactorial, leading to various clinical manifestations and phenotypes that relate to gene-environment interactions. Hundreds of genetic variants have been identified to be associated with the development of asthma[126,127], with heritability ranging from 35% to 95%[128]. However, the discordance of asthma in identical twins[129] suggests that genetics is not the only factor influencing the development of asthma. Indeed, among twins with no family history of asthma, environmental factors have been demonstrated to be sufficient to explain asthma development[130].

The increased prevalence of asthma is influenced by geographic variations, which supports the hypothesis that environmental exposure is an important factor in the development of asthma. Smoking is an important risk factor for asthma development[131], particularly in women[132]. Prenatal and postnatal passive exposure to smoke has been demonstrated to increase the risk of asthma development in children

at a range of 21% to 85%[133]. Childhood smoke exposure has also been associated with the incidence of adult asthma[134]. Moreover, exposure to indoor and outdoor pollutants is an important risk factor for the development of asthma[135]. The increased prevalence of asthma in urban communities[136] due to pest allergens[137], particulate matter, and nitrogen dioxide[136] has long been recognised as a risk factor for asthma[136], and interventions targeting pollutant exposure can reduce asthma morbidity[138,139]. Viral and bacterial upper respiratory tract infections (URTI) and lower respiratory tract infections (LRTI) play an important role in the development of asthma. Studies have consistently shown that early viral LRTIs are associated with the development of wheezing and asthma in children[140,141]. Other important risk factors include obesity, work environment and stress[132].

Heterogeneity of asthma

Asthma heterogeneity, first described by Francis Rackemann in 1927[142], has been a topic of interest in research for a long time, and considerable research effort has focussed on understanding and deconstructing the underlying disease process of asthma. Traditionally, the term "Asthma" was used to refer to a specific respiratory disease associated with a set of respiratory-related symptoms and clinical features, when in fact, such symptoms and features are not specific to asthma[143] and could easily be related to other diseases or syndromes. A significant flaw in this understanding was the assumption that asthma is a single entity with well-defined characteristics, which the new approach to defining asthma has proven to be an oversimplification[144]. The Lancet Commission on Asthma argued that such a notion of asthma is obsolete, and that the adoption of this approach led to "progression stagnation" in key outcomes over recent years[145]. A greater understanding of the pathophysiological processes underlying asthma has led to the description of various possible asthma subtypes called "phenotypes" and "endotypes"[146].

As opposed to the traditional allergic versus non-allergic classification system of asthma, phenotypes describe groups of patients with different clinical, physiological and biological characteristics, whilst endotypes describe different underlying pathophysiological mechanisms[29]. Under this perspective, asthma is mainly divided into two fundamental endotypes based on the presence or absence of ongoing type 2

inflammation: T2-high asthma and T2-low asthma. These endotypes underlie a variety of described asthma phenotypes. For instance, early-onset allergic asthma and aspirin-exacerbated respiratory disease are phenotypes of T2-high asthma, while obesity and smoking-related asthma are subtypes of T2-low asthma[147]. Such an approach to classifying asthma was based on the need for a comprehensive understanding that accurately addresses the heterogeneity of the disease and ultimately helps develop asthma management guidelines that optimise control over the disease and reduce the risk of severe outcomes. This appreciation of the complexity and heterogeneity of asthma has led to the realisation that a "one size fits all" treatment approach is not adequate and that a more personalised approach is needed[148].

However, in spite of the advancement in understanding the pathology of asthma, the increasing use of diagnostic tools such as FeNO and FOT, and the introduction of biomarker-based biological therapy, progression in asthma treatment discovery has been disappointingly slow[149], and asthma-related admissions are not decreasing despite increased spending on treatment[150].

Asthma Attacks

Definition, terminology, epidemiology, and disease burden

Definition of asthma attacks

There is a lack of consensus over the definition of asthma attacks and, as a result, these events are vaguely defined based on four main domains: worsening of symptoms, lung function deterioration[151], treatment received, and the need for medical attention, i.e. ED visit or hospitalization[152] (Table 1).

Table 1. Definition of exacerbation in asthma guidelines.

Guideline	Definition
ATS/ERS[152] Severe exacerbation Moderate exacerbation	 Events that require urgent action on the part of the patient and physician to prevent a serious outcome, such as hospitalization or death from asthma. Event that, when recognized, should result in a temporary change in treatment, in an effort to prevent the exacerbation from becoming severe.
GINA[151]	Progressive increase in symptoms of shortness of breath, cough, wheezing or chest tightness and progressive decrease in lung function, i.e. they represent a change from the patient's usual status that is sufficient to require a change in treatment.
WHO[153]	Episodes of progressive increase in shortness of breath, cough, wheezing, chest tightness, or a combination of these symptoms.

ATS/ERS: American Thoracic Society/European Respiratory Society. GINA: Global Initiative for Asthma. WHO: World Health Organization.

In 2009, the American Thoracic Society/European Respiratory Society (ATS/ERS) established a task force to provide recommendations on definitions of asthma outcomes, including AAs, primarily aiming to standardise the definition of endpoints for

clinical trials[152]. The task force defined moderate AAs as symptoms worsening characterised by a change in symptoms that is outside the usual daily variations, deterioration in lung function, or increased inhaler use for a minimum of 2 days, necessitating a change in treatment. The task force further defined severe AAs as the need for treatment with systemic corticosteroids (SCS) for a minimum of 3 days or the need for hospital attendance. Although the task force definition of AAs provides a clear overall insight aiding the recognition of these events with severity scales, there are limitations regarding its utility in clinical practice (understandably, as these definitions are intended to inform endpoints in clinical trials). Firstly, it does not acknowledge the utility of objective measures of airflow limitation such as peak flow or FEV_1 in the context of severe episodes and is mainly based on retrospectively made treatment decisions, i.e., treatment with SCS or hospitalisation/A&E visit. Moreover, the ATS/ERS severity scale for AAs doesn't provide a definition for milder episodes as it considers minimum symptoms and peak expiratory flow (PEFR) variations a "*transient loss of asthma control*", allowing a great deal of subjectivity in the recognition of symptoms of possible, impending AAs[154]. The FACET study looked into the pattern of PEFR and symptom changes preceding AAs in 425 patients and identified a gradual pattern of deterioration in the 14 days preceding an abrupt decline in lung function[155]; it is worth noting that findings from the FACET study are based on mean values and that there were still variations among patients in terms of changes in PEFR, symptoms, and reliever use. Further, AAs are characterised by a linear PEFR deterioration over an average of 9.6 days, with a diurnal variability pattern very similar to those observed during well-controlled periods, as described by Reddel et al.[156]. This essentially means that using the ATS/ERS criteria, recognition of AAs is difficult and only possible later on in the course of deterioration when symptoms become noticeable, missing a potentially valuable opportunity for early intervention, perhaps with inhaled corticosteroids (ICS)[157,158].

Similarly, the Global Initiative for Asthma (GINA) 2019[151] defines AAs as the worsening of symptoms along with the need to change treatment; it is worth noting that GINA's definition, commonly used in clinical practice, is largely based on that of the ATS/ERS, clinical trials-oriented, definition. However, GINA incorporates deterioration of lung function assessed using objective measures, such as PEFR and spirometry, as an

objective defining criterion. The World Health Organization (WHO) definition of AAs is centred around the deterioration of symptoms and lung function and does not account for associated treatment, the need for medical attention, or the degree of the severity of the episode[153].

Clinical trials adapting current definitions as endpoints require large sample sizes to power studies and, as a result, require longer duration. In an effort to address these issues and to facilitate recruitment for AA trials, Fuhlbrigge et al.[159] developed a prospective Composite endpoint for Exacerbations (CompEx) defining AAs for use as a primary endpoint in trials using prespecified criteria; these include changes in asthma-related variables (expiratory flow, symptoms, awakenings, and reliever use) OR a severe exacerbation defined using traditional criteria. By incorporating predetermined, objectively measured markers of deterioration, CompEx was able to capture almost thrice the events defined by the need for SCS alone, highlighting the potential utility of prospectively defined "biomarkers" of exacerbations.

Common to these definitions are the different viewpoints around the defining criteria of acute asthma, with the majority of these heavily depending on retrospective treatment decisions needed to manage these events. Prevention of asthma exacerbations is an imperative management goal in the guidelines[2,20,160], yet no consensus over objective, prospective defining criteria exists until now. This is a particularly important issue given the varying levels of perception of asthma symptoms severity and the significance of AAs, not only by patients but by clinicians as well[161-166].

A prospective defining criteria addressing the heterogeneity and the underlying inflammatory mechanisms involved would allow a precise, patient-specific treatment of AAs using personalised treatment options, such as those utilised in severe asthma; however, this is hindered by a poor understanding of the underlying complexity and mechanism of acute asthma, which has largely been managed in the same way since the 1950s[167-169]. There is a lack of knowledge regarding reliable, objectively assessed measures of physiological impairment and inflammatory processes in AAs, which has led to the widespread adoption of a "one-size-fits-all" notion of treatment with SCS and beta-agonists at the first sign of asthma-related symptoms deterioration.

Asthma control and attacks

Achieving good control is a core principle in the management of asthma and is based on the level of symptoms control and risks of AA[2]. However, the distinction between poorly controlled asthma and AAs is difficult, and events characterised by a decreased morning PEFR by 15%[170], 20%[171], nocturnal symptoms[172], or increased reliever use[173] have been referred to as mild exacerbations in some trials, despite ample evidence describing features of and differences between periods of poorly controlled asthma and genuine AAs[155,156,174].

Asthma control and exacerbations are closely related, and AAs are considered the "tip of the iceberg" of poorly controlled asthma. Poor control is a well-recognised risk factor of AAs[175], and good asthma control reduces the risks of AAs[176,177]; however, even those with fewer symptoms[178] and well-controlled disease[17,156] can have AAs; thus, it has been argued that asthma control and exacerbations could be driven by different underlying disease mechanisms[179].

Characteristics of poorly controlled asthma and AAs were delineated in an elegant study by Reddel et al.[156], who described symptoms and PEFR variations of 40 AAs occurring in 26 patients. PEFR values were collected daily during a 28-day poor control run-in period, the subsequent period of stable asthma after initiating inhaled budesonide, and during AAs. Patients were assessed every eight weeks for a total period of 18 months.

During the run-in period (poor control), the average diurnal PEFR variability (calculated as the daily amplitude percent of the mean) was 21.3%. This average fell to 5.4% during periods of well-controlled disease. During AAs, PEFR linearly fell and improved over an average of 9.6 days with 7.7% diurnal variability and poor bronchodilator response. Although PEFR variability was similar in periods of control and AAs, diurnal variability was higher, morning PEFR was lower (63.5% vs. 77.2%), and bronchodilator response was better (28% vs. 1%) during periods of poorly controlled asthma compared to periods of AAs.

Again, such difficulty in distinguishing between exacerbations and poor control is owed to the vague clinical definition of AAs and the lack of objectively assessed markers of inflammation during attacks and periods of poor control.

Terminology

Asthma attacks are significant events associated with long-term and permanent consequences[180,181] and, thus, warrant a concise use of terminology from both clinicians and patients. Unfortunately, the use of terms describing AAs is not standardised, and, as a result, studies have consistently highlighted confusion, misunderstanding, and differences when using terms to describe these events[163,182,183]. The Global Strategy for Asthma Management and Prevention Report 2021 (GINA) recommends using the term "*flare-up*" in clinical settings as it is more convenient for patients to use and remember compared to the term "*Exacerbation*" and implies the existence of the condition even when symptoms are under control[2]. The report does not recommend the use of the term *attack* as it suggests a sense of a sudden onset as opposed to a gradual deterioration. On the contrary, in 2014, the British guideline on the management of asthma advocated the use of the term *asthma attack* as it indicates the urgency and seriousness of the event[184]. Moreover, the Lancet Commission on Asthma 2017 states that the terms "*flare-up*" and "*exacerbation*" may not necessarily indicate the seriousness of acute asthma as they imply a less serious, easily reversed condition and that the term "*attack*" calls for prompt attention[145].

There is a lack of agreement regarding the terms used to describe AAs among guidelines, which has translated into different views and interpretations of these events by patients and clinicians, highlighting the importance of consistent use of terminology by both clinicians and patients.

Epidemiology and burden of asthma exacerbations

Asthma attacks, a common complication of asthma, are responsible for more than 400,000 deaths every year globally, with the majority of asthma deaths in middle-income countries[185]. Although there has been a noticeable decline in hospital deaths attributed to asthma in 2010 compared to 1990[12,186], there has also been a noticeable stagnation in key areas, including mortality and admissions, since 2000[145,187]. In the United Kingdom (UK), AAs are responsible for a significant number of hospital admissions and deaths[84]; the past decade has witnessed more than 30% increase in deaths attributed to asthma[188,189] and a subsequent high cost of care to the NHS[13]. It has been suggested that the stagnation of asthma mortality rates in the UK is due to

the significantly increased numbers of deaths in the older population (>75 years) as compared to the younger (≤75years), which has been speculatively explained by misclassification of causes of death due to other respiratory diseases, such as COPD, as asthma[190], supported by evidence from the National Review of Asthma Deaths report highlighting potential imprecision of the coding system[191].

Asthma attacks are accountable for a significant utilization of health care; the direct and indirect costs of asthma are the highest among non-communicable diseases in the United States and Europe and are mainly attributed to caring for AAs[15,192-194]. Moreover, the cost of care for exacerbating asthma patients is at least 40% higher than those with a stable disease[84], and those with a severe disease consume more than 60% of the cost of asthma care due to AAs[195]. Sub-optimally controlled asthma and the subsequent AAs place a substantial economic burden on healthcare systems[196-198].

Despite this, deterioration of symptoms and hospitalisation may only be "the tip of the iceberg" of AAs; the consequences of these events extend far beyond the course of symptom deterioration. In addition to being a risk factor for another attack[199-201], AAs are associated with permanent and irreversible loss of lung function[5,84,180], likely due to airway remodelling[8]. Further, AAs have a considerable effect on daily activities and psychological well-being[181] and lead to poor quality of life[202,203].

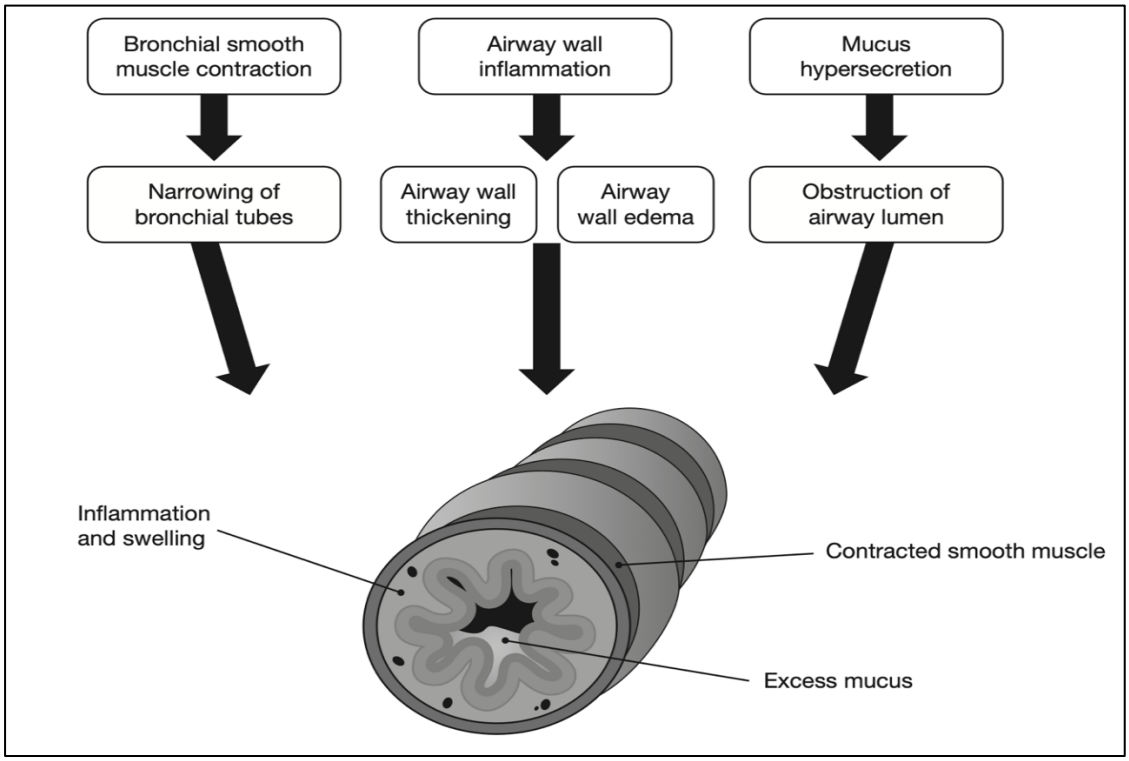
Unlike other diseases, where the unintended effects of the usual dosage of the treatment are referred to as adverse drug reactions[204], unintended effects of systemic corticosteroids for acute asthma are commonly referred to as *toxicity*[205] as they entail morbidities that can impair disease control, leading to further deterioration of asthma and hence, asthma attacks. These SCS-related toxicities include obstructive sleep apnoea[206], weight gain, depression, and increased risk of infections[207], all of which are considered risk factors for asthma attacks[208]. Studies suggest a threshold of 4 courses of systemic corticosteroids a year to be associated with toxicities[206,209]; however, a single SCS exposure may be sufficient to cause SCS comorbidities[206,210], and evidence indicates a dose-response association between SCS and adverse outcomes[211].

Pathophysiology and aetiology of asthma attacks

Pathophysiology of asthma attacks

The pathophysiology of AAs involves airway inflammation, airway smooth muscle contraction, and mucus plugging (Figure 3), all of which can lead to the symptoms observed during acute asthma episodes. It is likely that the pathophysiological processes responsible for AAs are heterogeneous depending on the underlying stable disease phenotype and triggers for attacks[212], but this is not well understood. Further, evidence suggest that airway remodelling and small airway dysfunction are important in the pathophysiology of acute asthma, however, their role and degree of involvement during AAs is not well understood.

Figure 3. Common mechanisms involved in the development of symptomatology in acute asthma.



Reproduced from Eid et al.[213].

Airway inflammation

Central to the pathophysiology of asthma is the inflammatory response to various triggers, which leads to the acute manifestations observed during symptoms worsening, including airflow obstruction, hyperresponsiveness, and mucus hypersecretion[214]. Such an acute inflammatory response seems highly dependent on the acute trigger[208]; however, it is not clearly understood whether the pattern of

inflammatory response is a reflection of the acute deterioration of the stable disease or a consequence of the acute trigger, but the latter is more likely given that exacerbation rates are reduced by controller treatments[215] but can still occur[171,216,217], even in those with mild[156] and well-controlled disease[17], and are not obviated by the same treatment used to optimise control and prevent exacerbations[218].

Rhinovirus (RV) is the most common trigger for AAs[219], and much of the knowledge of the role of viruses in AAs comes from studies investigating the immunopathogenesis of RV infections. IL-8-mediated neutrophilia is a common finding in AAs triggered by respiratory viruses[220,221], including RV[222,223]. IL-8 is a potent neutrophil chemoattractant[224] that is associated with airway hyperactivity and AA severity[223,225]. Experimental RV infections have been demonstrated to result in lower airway inflammation (both neutrophilic and eosinophilic), worsening respiratory symptoms, lung function impairment, and airway hyperactivity in asthmatics but not controls[226]. The inflammatory response to RV also includes the induction of IL-6, tumour necrosis factor-α, IL-β, RANTES, and granulocyte-macrophage colony-stimulating factors[227]. Although neutrophils and their by-products, including chemotactic factors, granule proteins, and neutrophil extracellular traps, are crucial for antimicrobial activity[228-230], neutrophils are linked to airway narrowing, mucus hypersecretion[231], and airway hyperactivity[232], all of which can occur during AAs[233].

Viral-induced T2-low activity has also been suggested to activate T2-high pathways[234,235], and RV is also linked to T2-high inflammation[236,237], which is known to play a key role in AAs[238]. Suppression of T2-high cytokines has been shown to reduce the rates of AAs[239,240], including viral-induced AAs[241]. NETosis, a product of neutrophil activation[242], has been demonstrated to augment T2-high response to RV through the induction of T2-high cytokine production, including IL-5[243]. Lower airway eosinophilia, paralleled with increased nasal IL-4, IL-5, IL-13, and IL-33, has been noted in RV16 experimental infections[244]; in this study, patients with asthma had greater respiratory symptoms and lung function impairment compared to controls, and IL-33 significantly correlated with lower airway symptoms and IL-5, suggesting ILC2 activation. The imbalance between ILC1 protection (delayed) and ILC2 inflammation (activated) in response to RV infection has been suggested to play a major role in the development of AAs[243].

Perhaps the strongest evidence of the implication of the T2-high pathway in the mechanism of AAs is that selective suppression of T2-high activity significantly reduces the rate of AAs (often defined as the need for a course of SCS)[236] but has a relatively modest effect on underlying disease activity, including lung function[245-247], symptoms[247,248], and airway hyperresponsiveness[247]. T2-high pathway susceptibility to AAs has been linked to the effect of T2-high inflammation on airway remodelling and the susceptibility to respiratory viruses[238]. Inflammation and airway remodelling mediated through IL-13 and IL-5 pathways predispose asthma patients to an amplified response to an insult[23]; exacerbations then further activate T2-high mediators that result in further airway remodelling, promoting subsequent AAs[249]. This is supported by evidence showing that AAs are associated with accelerated loss of lung function (baseline reduction of lung function)[84], which has been shown to be associated with frequent exacerbations[250,251]. IgE is associated with impaired interferon responses to viral stimuli[252,253], and evidence suggests that Omalizumab restores antiviral activity and reduces virus-induced AAs[241,254].

Although neutrophilia has commonly been attributed to viral exacerbated asthma[220,255], airway neutrophilia has been noted in non-infectious AAs[256] (likely confounded by systemic corticosteroid[257]). Unlike T2-high pathways, the role and extent of neutrophilic inflammation in the development of AAs is less well understood, although evidence suggests a degree of association[258,259]. Peters and colleagues investigated the utility of eosinophils and IL-6 in predicting AAs in exacerbation-prone compared to exacerbation-resistant patients. Although blood eosinophilia was associated with a higher AA risk in the whole cohort, 60% of the exacerbation-prone patients did not have blood eosinophilia, and IL-6, but not eosinophils, was associated with higher AA risk in the exacerbation-prone group even when restricting the analysis to those not on biologics or oral corticosteroids in the previous year[260]. Sputum neutrophilia is associated with steroid insensitivity[39] and disease severity[261]; however, although asthma is broadly classified into eosinophilic and neutrophillic[24], the Severe Asthma Research Program (SARP) study identified a cluster of patients with a severe disease that is insensitive to steroids characterised by both eosinophilic and neutrophilic airway inflammation[262], suggesting that a combination of the two pathways could also play a role in disease severity and steroid refractoriness. Moreover,

Th17 has also been demonstrated to be involved in steroid resistance and severe asthma[263], and sputum IL-17 has been found to correlate with sputum neutrophils and IL-8 in severe steroid-resistant asthmatics but not controls[264]. The aetiology of steroid refractoriness is poorly understood but has been linked to bacterial colonisation and decreased diversity of airway microbiota in the airway of those with severe corticosteroid refractory asthma[265]. It should also be noted that while severe asthma is steroid insensitive[266,267], corticosteroids have been demonstrated to delay neutrophil apoptosis[268,269], and airway neutrophilia has been associated with both inhaled and systemic corticosteroid exposure[257,270]. Altogether, there is a well-established but poorly understood interaction between different pathways that could underpin the association between viruses, neutrophils, steroid insensitivity, disease severity, and AAs. Nonetheless, as previously mentioned, the direct mechanism of the contribution of T2-low pathway in AAs is not clear, but evidence suggests that dysregulated IFN-γ response and the defective interferon-I immune response may play a key role[271].

Inflammation is also implicated in the remodelling of the airways[272,273], which has been suggested to predispose asthma patients to AAs[238,249,272] and has been shown to have a role in the clinical manifestations of AAs[233]. Holgate and colleagues argue that AA-associated damage and subsequent imperfect repair of the epithelium provides a "soil" involving the release of growth factors and cytokines, promoting sustained T2-high inflammation that further augments the remodelling process, a theory referred to as epithelial mesenchymal trophic unit (EMTU)[274,275]. Dougherty and colleagues suggest that "in this way asthma exacerbation may become self-perpetuating with one exacerbation causing changes in lung function that promote a subsequent exacerbation"[249].

Airway smooth muscle contraction

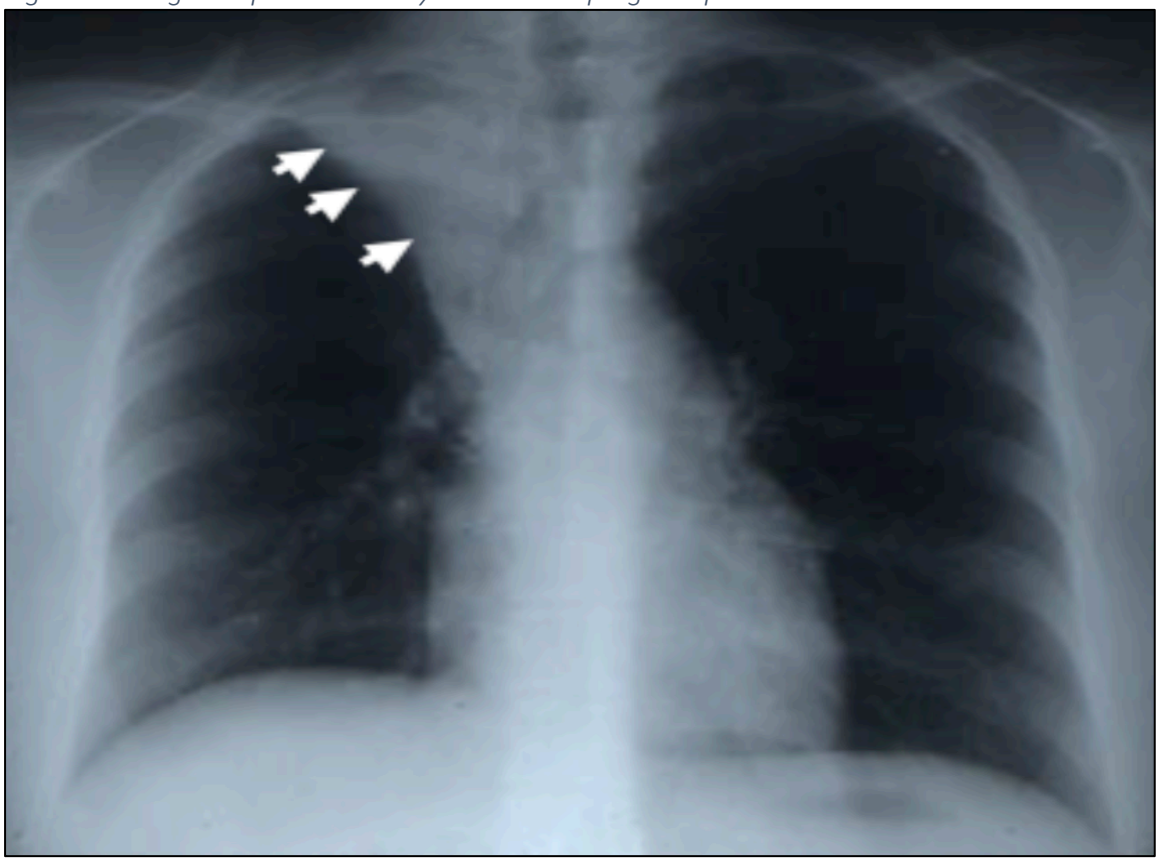
Airway smooth muscles (ASM) encircle the airways and play a key role during asthma attacks. ASM regulate the airway diameter, and contractility of ASM account for the airway narrowing observed during asthma attacks[276]. Airway hyperresponsiveness, defined as the increased susceptibility of the airways to excessively narrow in response to exposure to a stimulus that would typically have no impact in healthy individuals[277], is a consequence of the activation and the subsequent contractility of airway smooth

muscles[49]. Such a reaction of the airway smooth muscle is provoked by various factors, including methacholine, histamine, and exercise[278]. The mechanism of hyperresponsiveness is unclear but has been attributed to the increased presence of eosinophils and mast cell mediators in the airways of asthmatics[279], as well as increased ASM stiffness[280] and mass[281]. Impaired ASM relaxation, owing to the limited innervation of ASM[276], is also an important feature contributing to airway narrowing[49]. ASM relaxation is further impaired by the downregulation of the b-adrenergic relaxation mechanism due to atopy[282] and rhinovirus infections[283].

Mucus plugging

Air trapping-related hyperinflation secondary to mucus plugging of the airways is a common finding in studies of fatal and nonfatal AAs[249,284,285] (Figure 4). Koessler's report[286] indicated that the airways of those who died from asthma are characterised by intraluminal viscous, eosinophils-based mucus plugs and lung collapse. Mucociliary clearance is impaired during AAs[287], and intraluminal mucus significantly increases the degree of airway resistance independent of the degree of smooth muscle shortening[288]. Mucus from cases of fatal asthma is unusually viscid with a high concentration of mucin[289], and the degree of intraluminal narrowing caused by mucus is higher in fatal asthma compared to non-fatal asthma and controls[288].

Figure 4. Lung collapse secondary to a mucus plug in a patient with an asthma attack.



Reproduced from Fahy et al.[290].

Airway remodelling

Much of the existing knowledge of airway remodelling in AAs is based on and derived from the pioneering work of Koessler, who was the first to describe features of airway remodelling in fatal asthma[286]. Subsequent qualitative descriptions[291-294] have consistently confirmed the findings of Koessler, that is, the presence of massive airway remodelling in cases of fatal asthma. Airway remodelling is defined as the structural changes of the airways and is characterised by thickening and fibrosis of the airways, as well as muscle hypertrophy[295]. Airway inflammation is thought to be responsible for the remodelling of the airways[272,273], but this is not well understood, especially given that remodelling can occur early in the disease process and without apparent inflammation[296]. Although remodelling of the airway is a common manifestation of recurrent AAs[84], airway remodelling can also have a role in the clinical manifestation of AAs[233,297], and the magnitude of the airway narrowing to an insult may be influenced by the degree of the underlying airway remodelling[298]. Indeed, in a study assessing the utility of fractal geometry in quantifying the severity of airway remodelling, Boser et al.

found that, compared to controls, cases of fatal asthma were associated with a less complex bronchial tree[299].

Accumulated sputum in the airway of asthmatics is a well-documented feature[286,300] associated with the remodelling of goblet cells and submucosal glands[291] and the subsequent increased sputum production[300]. The consequences of such structural changes mainly involve mucus hypersecretion-induced airway narrowing[285,301]. Indeed, increased sputum production is common during AAs[302], and it has been suggested that acute degranulation of hyperplastic goblet cells may play an important role in the pathophysiology of AAs[303]. Hyperplasia of the goblet cells, the primary source of airway mucus[304], is commonly seen in the airways of asthma patients[305], particularly in fatal asthma[300,306], and leads to the hypersecretion of highly tenacious and viscous eosinophil-based mucus[307], and subsequent airflow narrowing[301].

It is not clear whether airway wall thickening can contribute to the pathophysiology of airway lumen narrowing during AAs, but a well-established direct association is demonstrated in stable asthma[308,309]. In a computer model of the airway assessing the relationship between airway thickening and airway resistance, Wiggs et al. demonstrated that airway wall thickening significantly augmented the degree of airway narrowing secondary to ASM shortening during constriction despite minimum effect at baseline[310]; the combination of airway wall thickening and loss of recoil were found to profoundly influence AHR. Moreover, James et al. assessed postmortem lung specimens to determine the effect of airway wall thickening on airway calibre narrowing and found that it takes 27% shortening of airway smooth muscle to achieve a 15-fold increase in resistance in asthmatics compared to 40% in the control group[311]. In contrast, evidence from in vivo high-resolution CT assessment of airway thickness indicates that the methacholine dose required to generate a 20% reduction in FEV_1[312], the level of FEV_1 reduction, and the degree of airway narrowing in response to methacholine challenge test[313] are not affected by the degree of airway remodelling and are similar between asthmatics and non-asthmatics.

In addition, cartilage degeneration has been suggested to increase airway collapsibility[314] and has been shown to augment the degree of airway narrowing in response to ASM contractions[273]. Emphysema-related loss of alveolar attachments and

decreased elasticity have been demonstrated to greatly influence the degree of airflow obstruction[315] and have been found to increase the risk of near-fatal AAs[316,317].

Remodelling of the airway can alter the properties of ASM that regulate the degree and velocity of ASM shortening[233], leading to airway narrowing[318], impaired ASM relaxation[319], and augmented shortening and contractility of ASM in response to stimulus[320]. It should be noted that airway wall thickness and the effect of ASM contractions on airway narrowing are closely related. Airway wall thickness decreases the load imposed by lung parenchyma on ASM, allowing for an increased ASM shortening and velocity, resulting in the closure of the airway prior to the end of exhalation[321]. Hyperplastic ASM, combined with thickened airway walls result in an augmented degree of airway narrowing in response to ASM shortening[311].

The small airways

Although it is not clear how SAD contributes to the pathophysiology and clinical manifestation of AAs, the balance of evidence tends to suggest a potential involvement of SAD in many of the aspects that can potentially contribute to asthma symptomatology. Shorter and colleagues[322] assessed nitric oxide concentration during exacerbation and resolution in asthma and COPD patients using a fast response infrared absorption system and found a tendency towards a decreased alveolar nitric oxide concentration at resolution compared to exacerbations in adult and children asthma patients. Kraft et al. demonstrated increased alveolar CD4+ number, decreased glucocorticoid receptor binding affinity and steroid responsiveness in nocturnal compared to non-nocturnal asthma. The number of alveolar, but not proximal airway, CD4+ cells significantly correlated with the percentage predicted FEV_1. The authors suggested that small airway inflammation may explain the impaired steroid anti-inflammatory effects and the resultant night-time exacerbations in patients with nocturnal asthma[323,324].

The extent of the small airways involvement in the clinical expression during AAs is not well-understood, but given their role in many of the elements of the asthmatic disease process, including symptoms and control, exacerbations, and AHR[325], it can be postulated that they can also play a role in the symptoms worsening during acute episodes. Wawszczak[326] and colleagues investigated the involvement of SAD in stable

and acute asthma in children and found that R_{5-20} was significantly higher in exacerbating compared to stable children. Wagner and colleagues, using a wedged bronchoscope technique, demonstrated an augmented small airway resistance response to histamine in asthma patients compared to controls[119]. Mansur et al.[327] evaluated the correlation between IOS (Impulse Oscillometry) indices and symptoms worsening following methacholine challenge test in asthma patients and found that R5 and X5, but not FEV_1, correlated with post methacholine symptoms, including chest tightness, dyspnoea, and wheeze, implying small, rather than large, airways involvement. Air trapping has also been shown to be associated with increased symptomatology in response to MCT[328], even after adjusting for FEV_1 reduction in a multivariate analysis[329,330]. The severity of AHR is also associated with the degree of SAD. Asthma patients (matched for FEV_1% predicted) with severe AHR (PC20 ≤1 mg/mL) have significantly lower $FEF_{25\%-75\%}$ compared to those with less severe AHR (PC20 ≥8 mg/mL)[124], and distal airway inflammation (bronchoalveolar lavage eosinophils) correlates well with the degree of AHR[331]. The small airways can also have a role in the response to allergens. Zeidler and colleagues[332] induced a late asthmatic response by exposing 10 asthma patients to cats, causing a minimum of 20% reduction in FEV_1, followed by spirometry and HRCT at 6 and 23 hours after the allergen challenge. Although FEV_1 returned to normal at 6 hours following exposure, $FEF_{25\%-75\%}$ was still significantly impaired at 6 hours and didn't return to normal until 23 hours following exposure. Air trapping and small airway hyperresponsiveness (assessed using HRCT image analysis), but not large airway hyperresponsiveness (as measured using PC_{20} FEV_1), persisted until 23 hours after allergen exposure.

Importantly, post-mortem studies of cases of fatal asthma have demonstrated altered mechanics of the lung periphery. Saetta and colleagues[333] analysed the peripheral airways and pulmonary arteries in autopsy lungs from patients with sudden fatal asthma compared to those who died from non-respiratory related conditions. The bronchioles of those with fatal asthma had significantly greater lumen narrowing, inflammatory infiltrates, mucus plugging, and ASM thickness. Further, pulmonary arteries proximal to the bronchioles had marked inflammatory infiltrates but lacked features of chronic hypoxia. The authors highlighted the potential role of such changes in the gas exchange abnormalities during cases of fatal asthma. More recently,

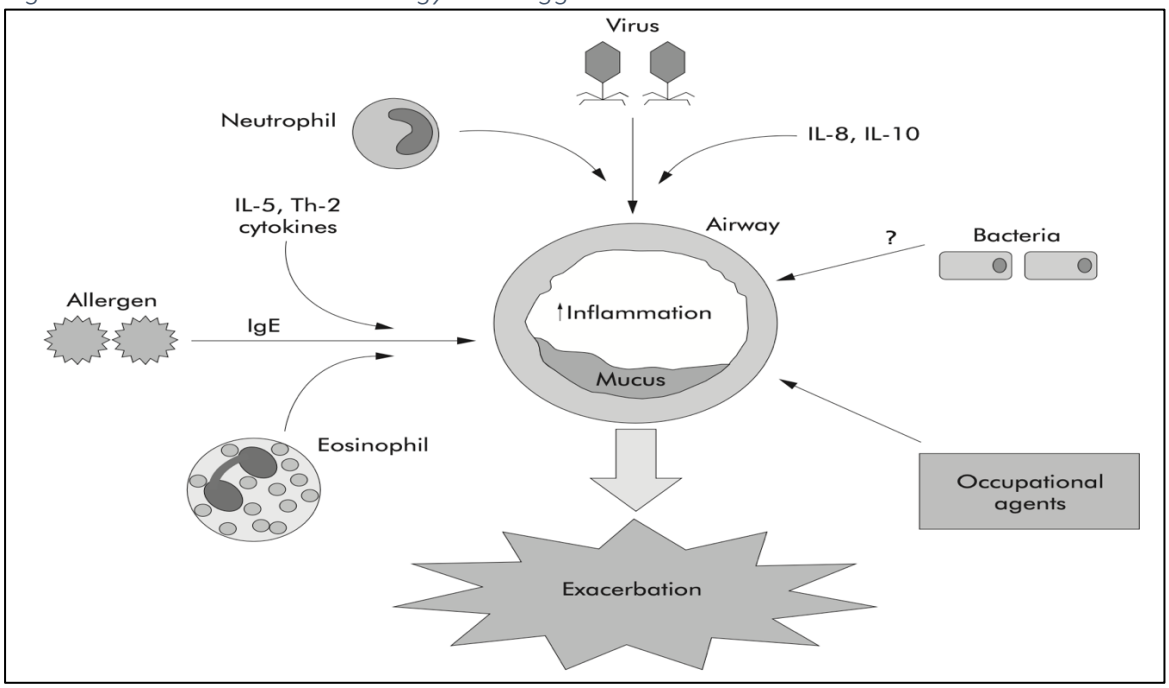
Dolhnikoff et al.[334] evaluated extracellular matrix (ECM) remodelling in the lung periphery and demonstrated profound ECM changes at the outer parts of the small airway walls; authors highlighted the potential consequences of such changes on airway parenchyma loss in patients with fatal asthma.

Although it is perhaps anticipated that the lung periphery may play a role during acute episodes of asthma, the extent of such involvement has not been thoroughly investigated.

Aetiology of asthma attacks

A wide range of factors can precipitate AAs (Figure 5), including viruses, allergens, bacterial infection, and pollution; however, the majority of AAs are related to respiratory viruses, most commonly rhinoviruses. The degree to which these contribute to the mechanisms underlying AAs and, importantly, interact with each other is not well understood.

Figure 5. Asthma attacks aetiology and triggers.



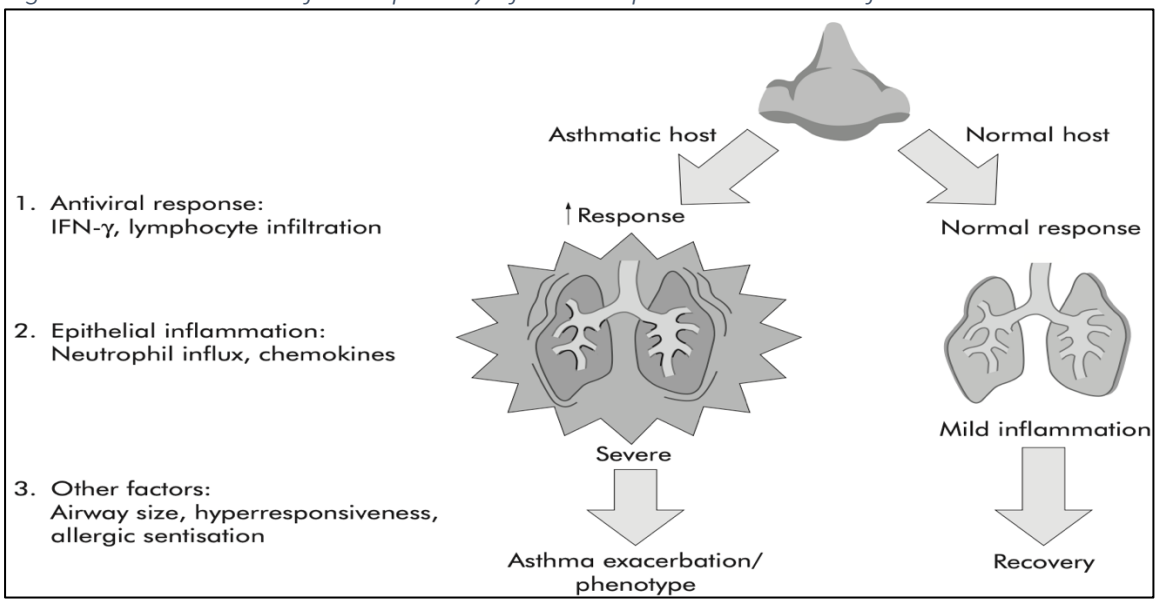
Reproduced from Singh et al.[335].

Respiratory viruses

Viral respiratory tract infections are responsible for about 80% of AAs[82], with rhinoviruses being responsible for the majority of these in adults and children[219].

Asthma patients are more severely impacted by rhinoviral infections compared to healthy individuals[244,336], and a strong correlation is noted between RV load and exacerbation severity[226]. In a recent analysis of near-fatal AAs requiring extracorporeal membrane oxygenation, over half the admissions were attributed to respiratory infections, with RV detected in the majority of them[337]. The mechanism underlying the relation between viruses and AAs is not well understood but is likely to be attributed to two interrelated mechanisms: the infection-induced inflammatory response and the impaired immune response to RV[249,338] (Figure 6).

Figure 6. Mechanisms of susceptibility of asthma patients to virus infections.



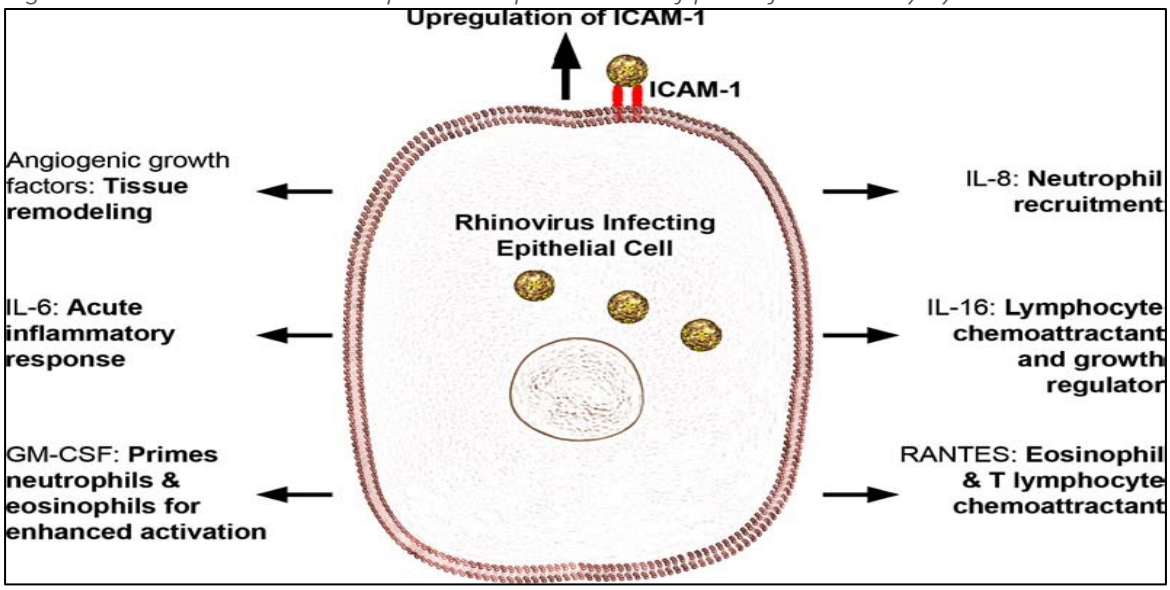
Reproduced from Singh et al.[335].

Neutrophils and IL-8 are commonly detected in the sputum, bronchial alveolar lavage, and nasal fluids of experimentally RV-infected asthma patients[82]. RVs have the ability to exist and replicate in the lower airway epithelium[339-342], which, consequently, induces the production of pro-inflammatory mediators, including IL-6, IL-16, IL-8, IL-1 and RANTES and subsequent bronchial cellular infiltration (Figure 7); such inflammatory response is thought to be responsible for the symptomatology of AAs[343,344]. Several of the abovementioned cytokines have a direct relationship with respiratory symptoms during RV infections[345]. IL-8 has been implicated in increased airway hypersensitivity[223], and IL-1 was found to augment ASM contractility and attenuate bronchodilator responsiveness of ASM[346]. IL-1 has also been found to correlate with symptoms severity

in adults experimentally infected with RV[347]. RV has also been shown to induce T2-high cytokines, including IL-25, IL-33, IL-4, IL-5, and IL-13[348]. IL-33 is important for the inflammatory response to RV and has been shown to correlate with viral load and AA severity[244]. Similarly, ILC2 has been shown to correlate with RV infection duration and exacerbation severity[243].

The susceptibility of asthma patients to RV has been attributed to defective interferon production and the impaired anti-viral response and subsequent increased viral replication[349]. Indeed, studies have consistently demonstrated a delayed or deficient interferon response to RV infections in asthma patients[350,351]. Such deficiency appears to be closely related to T2-high pathways; however, this is not well understood. Altman and colleagues demonstrated that although both baseline increased T2-high inflammation and deficient interferon type I response in asthma children predict short-term (30 days) exacerbation (both viral and non-viral) risk, the ratio of T2-high inflammation to IFN I response further increased the exacerbation probability[352]. Using a murine model, Duerr et al. demonstrated that deficient IFN response is associated with dysregulated ILC2 activation and T2-high associated immunopathology, suggesting that IFN response plays a key role in the regulation of T2-high pathways[353].

Figure 7. Rhinovirus-induced epithelium production of pro-inflammatory cytokines.



Reproduced from Friedlander et al., 2005[344].

Bacterial infections

The role of bacterial infection in AAs is less well understood compared to viral infections and involves complex interactions with respiratory viruses. As with viral infections, asthma patients are more susceptible to bacterial infections compared to healthy individuals[354,355], and induction of IFN-λ in response to bacterial infections is significantly impaired in asthma patients compared to healthy individuals[350]. However, the prevalence of bacteria-induced AAs is not clear, although the relationship between AAs and atypical pathogens is well-established[356]. In a study comparing the rates of atypical infections between patients hospitalised with AAs and a non-asthma hospitalised control group, Mycoplasma pneumoniae was significantly associated with hospitalisation for AAs, with a respiratory virus co-infection detected in the majority of those with Mycoplasma pneumoniae infection[357]. Similarly, serologic evidence of Chlamydia pneumoniae has also been found in 38% of patients presenting to the emergency department for AAs; those with Chlamydia pneumoniae had significantly higher sputum neutrophils compared to those without[358]. Chlamydia pneumoniae induces the production of several cytokines associated with respiratory symptomology[223,346,347], including IL-1 and IL-8[359-361], and is associated with sustained inflammation and airway hyperresponsiveness[362].

Several studies suggest a viral-bacterial interaction involvement in the development of AAs[363]. In-vitro evidence has demonstrated a virus-induced impaired macrophage antibacterial response[364], and a co-existing viral/bacterial infection has been observed during AAs[365]. Kloepfer and colleagues assessed bacterial and viral detection using viral diagnostics and quantitative PCR in children with asthma compared to controls during RV season and found that the rate of bacterial detection was significantly higher in children with asthma compared to those without; the combination of RV and a bacterial pathogen was associated with increased symptoms and risk of AAs[366].

Allergens

Environmental factors can have an important role in the development of AAs. There is a well-established association between allergen exposure and risk for asthma exacerbations[367], asthma-related hospitalisations[368,369], and even death[370]. Common allergens include dust mites, pollen, cats, cockroaches, and mould[367-369]. In addition,

thunderstorm-related exposure to airborne allergens has also been linked to an increased number of acute symptoms requiring emergency treatment[371]. As in viral and bacterial-induced AAs, allergen exposure induces an inflammatory response, leading to worsening of asthma symptoms[335,372]. The inflammatory response to allergens is predominantly eosinophilic, driven by IL-5 and IL-13, with a late asthmatic response demonstrated by the presence of T2-high cytokines 48 hours after antigen exposure[373]. There appears to be a synergistic mechanism between allergen sensitization and viral infections triggering AAs[374,375]. The risk for asthma admission is considerably higher in patients who are sensitised and exposed to an allergen with concurrent viral infection compared to those with an allergen exposure alone[376,377]. Atopic asthma is associated with impaired viral clearance, reduced IFN responses, and increased viral replication[349,378,379]. Targeting immunoglobulin E (IgE) with Omalizumab in atopic children has been shown to enhance IFN responses and improve asthma outcomes, including exacerbations, suggesting an important role of IgE-mediated inflammation in anti-viral responses[241].

Risk factors, comorbidities, and mimics for asthma attacks

A recent AA is the strongest predictor of a future one[199]. The mechanism underlying this relationship is not well understood but may be attributed to the *late asthmatic response* characterised by airway hyperactivity and persistent airway inflammation in response to viral-induced AAs[380-382]. Nonetheless, sub-optimal disease control and poor medication adherence are known to increase the risk of AAs[176,383]. Increased asthma severity is suggested to be associated with increased risk of AAs[384], although people with milder disease can still have frequent and even life-threatening AAs[199,385]. Female gender[386], older age[387], and black ethnicity[388] have also been reported to increase the risk of AAs.

Comorbid diseases are important contributors to AAs, and those with frequent exacerbations have been reported to have multiple comorbidities compared to less frequent exacerbators[389,390]. Among the most important contributors are gastroesophageal reflux disease (GERD) and chronic sinusitis[390]. Both of these conditions are independently associated with the risk of AAs, and it has been suggested that microaspiration of oral and upper airway secretions resulting from these

conditions[391] can contribute to the risk of AAs[389]. In addition, obstructive sleep apnoea is an important contributor to nocturnal AAs[390], and improvement in asthma outcomes has been observed following continuous positive airway pressure therapy[392]. Further, smoking[250,393], obesity[384], recurrent respiratory infections, and depression[390] are all well-established risk factors of AAs.

Many diseases can mimic the presentation of acute worsening of asthma symptoms. Among the well-known asthma mimics are inducible laryngeal obstruction (ILO, previously referred to as vocal cord dysfunction) and breathing pattern disorder (BPD, also known as dysfunctional breathing).

Inducible laryngeal obstruction is defined as "adduction of the vocal folds, mainly during inspiration inducing dyspnoea of varying intensity"[394] ILO, previously termed hysteric croup[395] and Munchausen's stridor[396], has long been identified but is gaining more interest in recent decades. The incidence of ILO in patients with asthma has been estimated to exceed 25%[397-399] depending on the diagnostic technique utilised, and 35% in symptomatic asthma patients[400], 2% in those presenting to ED with asthma-like symptoms[401], and 18% in those with severe episodes requiring hospitalisation[402]. Such variation in reported prevalence may be attributed to the different sample population studied and, most likely, the overlap of symptoms between the two conditions[403] and the potential for both diseases to co-exist[404].

Laryngeal hyperresponsiveness is thought to be the main pathophysiological process underlying ILO, driven by a damaged laryngeal mucosa[394] resulting from different comorbid diseases including post-nasal drip[405], laryngopharyngeal reflux[406], rhinosinusitis[407], and GERD[408]. ILO is laryngoscopically diagnosed when a minimum of 50% larynx closure occurs in response to a provocation challenge testing[409]. Performing such a diagnostic technique during an acute presentation with worsening symptoms can be difficult; flow-volume loop assessment has been demonstrated to be unreliable for the diagnosis of ILO[404,410].

Breathing pattern disorder, also referred to as dysfunctional breathing, functional breathing disorder, or hyperventilation[411,412], is defined as the chronic changes in the pattern of breathing resulting in respiratory and non-respiratory symptoms that are not explained by a diagnosis of medical conditions such as asthma or other respiratory diseases[413]. Hyperventilation syndrome, first described by Kerr and colleagues in the

1930s[414], is the most commonly used term describing breathing pattern disorders, although hyperventilation is only one of the different types of BPD and not all patients with BPD have a hyperventilation breathing pattern.

The prevalence of BPD is difficult to estimate owing to the lack of gold standard diagnostic criteria and, most importantly, the lack of a consensus over a formal definition of the disease. The Nijmegen questionnaire is the most commonly used diagnostic method, although its sensitivity for the diagnosis of BPD has been questioned and the need for objective measures of diagnosis has been highlighted[415]. In addition, although validated in patients with asthmas[416], it has been suggested that the Nijmegen questionnaire is likely to overestimate the true prevalence of BPD given the overlap of symptoms assessed by the questionnaire and symptoms of asthma[413]. Nonetheless, the reported prevalence of BPD in the asthma population, assessed using the Nijmegen questionnaire, ranges from 25% to 47%[417-419].

Breathing pattern disorder involves a dysregulated rate or depth of breathing, including hyperventilation, hypoventilation, or disordered breathing mechanism involving the oropharynx, larynx, nasal passages, diaphragm, or chest wall muscles[420]. BPD results in asthma-like symptoms, including chest tightness and dyspnoea[413,421]. Although the prevalence of BPD during acute episodes of asthma has not been previously reported, BPD is known to mimic asthma symptoms and confound clinical assessment[422]. In a study describing the incidence and risk factors for BPD in asthmatics at a specialised asthma centre, 16 (18%) of the patients had BPD (confirmed using progressive exercise test)[415]; those were likely to be females with uncontrolled asthma, psychopathology, GERD, rhinitis, and frequent asthma exacerbations (defined as ≥3 exacerbations requiring systemic steroids, ED visit, or hospitalisation for asthma in the previous 12 months) compared to asthmatics without BPD[415]. The pathophysiology of BPD is not well understood but is thought to be linked to psychiatric diseases[413], although associations with common asthma comorbidities, including sinonasal diseases and GERD, have been previously reported[415,417].

Given the well-documented coexistence of multiple, often not mutually exclusive[404,423], comorbidities in asthmatic patients, and the hypothesised potential (or likely) interaction between them[417,424], it has been argued that the classical approach targeting asthma control and risk of AAs is not sufficient to improve asthma

outcomes[425]. The *precision medicine* based *treatable traits* model, is hence an attractive approach for the management of asthma exacerbations.

Precision medicine and the treatable traits in the context of asthma attacks

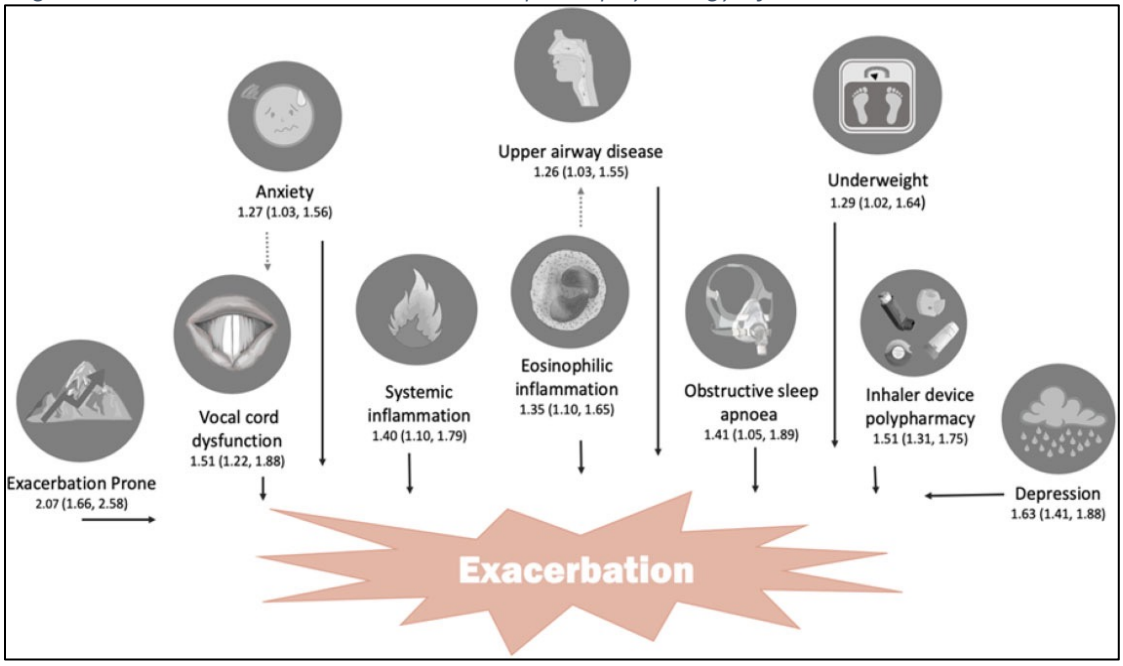
The recognition of the complexity and heterogeneity of chronic asthma has led to the realisation that a personalised precision medicine approach of treatment, as opposed to the one-size-fits-all approach, based on treatable disease traits is much needed[148,426]. Precision medicine is a treatment approach that targets the distinct needs of specific individuals based on biomarkers, phenotypes, and other characteristics that differentiate a specific patient from others with similar characteristics, with an overall goal of optimising clinical outcomes and minimising unnecessary side effects[427]. The term treatable traits can be defined as the identifiable, modifiable, and relevant clinical characteristics of a disease[426]. A treatable trait refers to therapeutic targets (phenotypes and endotypes) identified through well-established biomarkers and involves a multidimensional evaluation of the presence of disease traits, including clinical history, risk factors, lung function, and inflammatory parameters, followed by a precise, personalised management approach independent of the disease label; the aim is to ensure a personalised and targeted management approaches.

The treatable traits (TT) approach was initially proposed for the management of chronic airway diseases by Agusti et al. in 2016[148]; however, in the Lancet Commission on Asthma, Pavord et al. argue that as information about airway diseases during stable periods can predict patterns of inflammation during acute episodes, the TT approach is thus "just as applicable" during acute AAs[145]. Similarly, Gibson et al. conducted a literature review aiming to explore the applicability of TT in the management of exacerbations of airway diseases and argue that the principles of TT "easily fit within management of acute exacerbations[423]." Indeed, T2-high inflammation is intensely involved in the pathophysiology of AAs[238], and targeting T2-high inflammatory markers has been shown to reduce the risk of AAs[428,429].

Many of the risk factors, comorbidities, and mimics mentioned above are considered treatable traits that have been argued to have a role during exacerbations of airway diseases (Figure 8)[423]. Eosinophilic airway inflammation is a well-known asthma treatable trait[426]. In a recent proof of concept study[430], Bafadhel et al. conducted a

randomised control trial targeting the eosinophilic endotype, irrespective of diagnostic labels, in patients with a physician-diagnosed exacerbation of asthma or COPD. Patients with blood eosinophils of ≥0.3 X10^^{9}/L were randomised into a 30 mg prednisolone for 5 days and Benralizumab injection once, Benralizumab once and a daily placebo tablet for 5 days, or a 30 mg prednisolone for 5 days and a placebo injection once. The primary outcomes were treatment failure over the 90 days period following treatment and symptoms burden measured using visual analogue scale at day 28 in the Benralizumab treated group compared to the prednisolone alone treated group. The Benralizumab treatment group had a significant reduction in treatment failure and fewer symptoms compared to the prednisolone-alone-treated group. Although a few limitations have been noted in the design of the study, including the use of a blood eosinophilia cutoff that can be argued to be within the normal range for asthmatics and the inclusion of patients with frequent exacerbations suggesting poorly disease control, this sufficiently powered landmark study for the first time demonstrated the utility of the treatable traits approach in the management of exacerbations of airway diseases. The broader utility of such an approach, however, is likely to be hindered by the lack of readily available and reliable markers of inflammation during acute episodes of asthma and the poor understanding of the acute pathology of the disease.

Figure 8. Treatable traits involved in the pathophysiology of acute asthma.



Reproduced from McDonald et al.[423].

Frequent exacerbators

Frequent exacerbators, or exacerbation-prone are terms commonly used to describe a group of patients with recurrent AAs, usually more than one AA a year. The exacerbation-prone phenotype is considered a unique intrinsic phenotype of asthma, commonly of greater disease severity and with risk factors related to access to care, inadequate disease control[431], gender[432], asthma comorbidities and mimics[433]. Other studies found that susceptibility to AAs in this group of patients is significantly associated with a recent severe AA irrespective of other clinical and demographic characteristics or disease severity and control, further highlighting the uniqueness of this subgroup and suggesting that AAs in this group are driven by mechanisms different than those involved in other characteristics of asthma[434]. Although frequent exacerbations have been closely linked to disease severity, patients with mild disease may also be prone to frequent exacerbations. The National Heart, Lung, and Blood Institute's Severe Asthma Research Program found that patients with frequent AAs had varying asthma severity ranging from mild (5%) to moderate (13%) and severe (54%)[435]. Studies also indicate that those with frequent AAs are at greater risk for severe AAs despite higher intensity treatment compared to single time exacerbators[251]. Described characteristics of frequent exacerbators include chronic sinusitis, non-steroidal anti-inflammatory intolerance, irreversible airflow limitation, reduced reversibility, high-intensity asthma treatment[251], psychological dysfunction, obstructive sleep apnoea[390], obesity, blood eosinophilia, female gender, GERD[432], elevated FeNO, and sputum eosinophilia[389,436].

Fahy and colleagues argue that exacerbations in this group of patients could be due to ILO or aetiologies other than asthma[249]. Indeed, many of the comorbid diseases noted above can contribute to ILO[437], which has been associated with frequent AAs[433,438]. Studies investigating abnormal vocal cord behaviour in asthmatics can be traced back to the 1980s[439], but more recently, Low and colleagues investigated laryngeal movement using CT larynx in 46 difficult-to-treat asthmatics and found that 56% of 32 patients with bronchodilator response indicative of asthma had aberrant vocal cord motion during either inspiration or expiration with frequently observed contraction and complete closure of the entire larynx[440]. Interestingly, an intermittent abnormal vocal

cord behaviour was noted in one of the participants who had normal vocal cord motion at the initial study visit but abnormal vocal cord behaviour at periods of breathlessness. The authors suggest the potential for asthma and ILO co-existence, rather than ILO being an alternative entity mimicking asthma, a valid hypothesis given the well-documented unusual laryngeal function[439] and laryngeal hyperresponsiveness in asthma patients[441].

However, descriptions of acute episodes or periods of increased symptoms in frequent exacerbators are scarce. Jain and colleagues investigated the incidence of asthma misdiagnosis in frequent exacerbators (requiring ≥2 emergency room attendances or hospitalizations); a rigorous diagnostic algorithm, including spirometry, methacholine challenge test, and CT imaging, was used to assess evidence of airflow obstruction and alternative diagnoses in 333 asthma and COPD patients over a period of 10 months. Among 171 frequently exacerbating asthmatics, evidence of airflow obstruction was established in 65% of the patients. In 60 (35%) asthma patients with no evidence of airflow obstruction, 11 (18%) had evidence of ILO, suggesting that such patients may have been symptomatic due to non-asthma pathology[438].

In addition, in one of the first systematic descriptions of patients with ILO, Newman and colleagues described the characteristics of 95 patients hospitalised over a seven-year period from 1984 to 1991. Fifty-three of the patients had asthma as well. Newman described a predominantly female group of patients with ILO misdiagnosed as asthma for more than four years. These patients had been on intensive asthma treatment, including oral steroids, and required extensive healthcare utilisation, averaging nearly ten emergency room attendances and six hospital admissions a year, with a significant proportion needing intubation on several occasions[433].

Heterogeneity and stability of asthma attack inflammatory phenotypes

Given the well-established heterogeneity of asthma as a stable disease, AAs are likely to be as heterogenous. However, although this is well recognised, it is still poorly understood and has not translated into clinical practice with guidelines mainly relying on a one-size-fits-all treatment approach of OCS as the main and only treatment option despite ample studies highlighting the heterogeneity and instability of the inflammatory mechanisms underlying acute asthma.

The association between asthma and eosinophilia has long been recognised[442]. Trials studying inflammatory markers during acute asthma go back to 1979, when Charles et al. found an increased number of eosinophils in the blood of patients with acute asthma[443]. In the same year, a Brazilian study performed a differential cell count of sputum samples and found significantly increased eosinophil levels in the samples from asthmatics compared to those from patients with chronic bronchitis, emphysema, and other pulmonary diseases[444]. Subsequently, in the 1980s, Baigelman and colleagues obtained and analysed sputum samples from 11 non-infective AAs and found that eosinophils accounted for more than 25% of the cells; no details about other cell types were reported[445]. Similarly, later on in 1995, Turner reported that 53% of the mild AAs investigated were associated with airway eosinophilia (sputum differential ≥4%); those had significantly increased airflow obstruction and hyperresponsiveness compared to subjects with no evidence of eosinophilic inflammation; 47% of the samples were neutrophilic, despite the absence of obvious viral infections. Causes for variation in cell counts, including atopic status and allergen exposure, were not different between the eosinophilic and non-eosinophilic groups[446]. In another study aiming to assess changes in inflammatory markers in sputum samples of 10 patients hospitalised with asthma, exacerbations were mainly eosinophilic with increased IL-5 levels[447]; improvement in FEV_1 and IL-5 correlated well with improvement in eosinophil levels. These events were presumed to be caused by cold, medication discontinuation, or undertreatment. Histological studies have also noted increased eosinophils in the bronchial sub-mucosa of fatal asthma cases[294,448].

On the contrary, in a study assessing inflammatory profiles in non-smoking patients with persistent symptoms suggestive of poorly controlled asthma, the authors concluded that 59% of the sputum samples were neutrophilic, IL-8-mediated in nature[449]. In another study involving severe acute episodes requiring emergency visits, predominant neutrophilic inflammation and increased expression of IL-6 and IL-8 were noted in sputum samples of 18 patients; half the patients reported having a recent chest or head cold, although this was not tested for[220]. In addition, bronchial lavage samples from 16 patients mechanically ventilated for status asthmaticus also show predominant neutrophilic inflammation with increased IL-8 levels; only two of these events were precipitated by a respiratory tract infection, and all had received treatment

with OCS before mechanical ventilation[256]. In another similar study, analysis of 10 tracheal aspirates from intubated asthma patients showed IL-8-driven, increased neutrophil levels. However, in this study, only 3 of those patients had been on systemic corticosteroids prior to hospital admission, and the number of neutrophils was not different between those with pre-SCS treatment and those without. Interestingly, the number of neutrophils in this study strongly correlated with the duration of mechanical ventilation, suggesting a potential role for neutrophils in the severity of those episodes[225].

Viral-induced AAs are often associated with neutrophilia. Norzila et al. analysed sputum samples of 38 children presenting to the emergency department for acute asthma and reported prominent neutrophilic inflammation and increased IL-8 levels, although IL-5-mediated eosinophils were also observed, suggesting a mixed inflammatory response. About 70% of the AAs were attributed to upper respiratory tract infection although this was not objectively tested for[450]. Moreover, in vivo analysis of nasal aspirates in children with confirmed viral-induced AAs provided evidence of an important role of IL-8 and the resultant neutrophilic inflammation[255]. In addition, post-mortem studies have also shown viral-associated, predominant neutrophilic inflammation in patients who died from fatal asthma[451].

A biological clustering analysis of AAs, defined by symptoms worsening necessitating a course of OCS, using sputum mediators, clinical features, and airway microbiome identified three distinct clusters of AAs[452]. The first cluster (7/32, 21.9%) comprised of bacterial-associated AAs characterised by blood and sputum neutrophilia and higher proinflammatory mediator levels, proteobacteria, and proteobacteria/firmicutes ratios. The second cluster (10/32, 31.3%) had increased sputum eosinophils, BEC, T2-high mediators, microbiome diversity, and bacteroidetes proportion. Increased T2-low mediators and proportions of actinobacteria and firmicutes were observed in the third cluster (15/32, 46.9%). Another clustering attempt of 825 patients hospitalised for AAs identified three significantly heterogeneous clusters. Cluster 1 (526, 63.8%) was primarily eosinophilic, with a better prognosis and response to SCS. Patients in cluster 2 (97, 11.8%) had increased eosinophils, neutrophils, IL-6, CRP, longer hospital stay and SCS insensitivity. In cluster 3, 202 (24.5%), patients were mainly neutrophilic with increased levels of IL-6 and CRP and an increased proportion of positive sputum for

bacteria and fungus; patients in this cluster had the longest length of hospital stays and higher numbers of ICU admissions[453]. In a similarly designed study, four distinct clusters were identified; however, patients in this study were assessed at least three days after admission when they were deemed well enough to undergo study assessment; hence, findings from this study reflect the recovery, rather than the acute, phase of AAs[454].

Moreover, in the MEX study, half the AAs in severe asthmatics receiving Mepolizumab were T2-high (sputum eosinophils ≥2%), with no apparent difference in symptoms score increase and lung function decline at exacerbation compared to baseline between the two groups, suggesting that heterogeneity exists even when T2-high activity is well supressed[455]. In contrast to these findings, Poznanski et al. demonstrated that AAs in sub-optimally controlled severe asthmatics receiving Benralizumab are largely neutrophilic in nature[456]. Less invasive biomarkers have also been used to differentiate between different inflammatory processes underlying AAs. In a more recent study involving severe asthma patients, AAs were predominantly T2-high (defined as FeNO> 20 ppb or BEC >150 cells/µL) with similar patterns of lung function deterioration and symptom scores compared to the T2-low AAs (FeNO ≤20 ppb and BEC ≤150 cells/ µL)[457].

Although the pattern of airway inflammation response to certain triggers can be predicted, i,e. viral induced AAs generally result in neutrophilic inflammation and allergen exposure results in eosinophilic AAs[214], the inflammation profile of AAs in an individual asthma patient can vary between consecutive episodes and hence may not be predicted from the stable disease phenotype or from previous exacerbation patterns. The inflammatory pattern in asthma appears to be of a dynamic and unstable overall nature although this is not well understood, and studies show conflicting results[429,458,459]. In a study periodically assessing inflammatory phenotypes over a one-year period, 42.3% of the patients had different inflammatory profiles at 4 monthly follow-up visits compared to those observed at baseline[460]; inflammation-based phenotyping (based on induced sputum eosinophils and neutrophils) were less stable compared to those based on physiological parameters including baseline lung function and reversibility. In the context of AAs, inflammatory phenotypes are not associated with the stable disease phenotype nor with the inflammatory pattern observed during previous acute episodes in a T2-low enriched cohort[457]. Further, in the MEX study,

almost 30% of the severe asthma patients had different inflammatory profile at exacerbation compared to baseline; unfortunately, however, although 21 patients had a second exacerbation and 6 had a third exacerbation, inflammatory profiles of repeat AAs were not reported. Further, in a study examining the inflammatory profiles in sputum samples obtained from 79 patients during 186 consecutive exacerbations of airway diseases including asthma, almost 50% of the patients had inflammatory profiles that are different than those observed in the previous episode; patients with a previous sputum eosinophilic or neutrophilic episodes were more likely, compared to those with a mixed or an unclassified phenotype, to have an unchanged inflammatory profile in the subsequent episode[461]. Further, in a randomised trial assessing the effect of Mepolizumab on clinical outcomes in T2-high asthma patients refractory to oral steroid treatment, one of the subjects in the placebo arm had three AAs, two of those were neutrophilic, the third was eosinophillic[462].

Despite variation in the definitions of AAs and different cutoff points for biomarkers measured, studies have consistently demonstrated widely varying and heterogeneous mechanisms underlying inflammatory process in AAs across different disease and episode severities.

Biomarkers and asthma attacks

A biomarker can be defined as a measured characteristic of a disease that can indicate the underlying biological and pathogenic processes, and response to treatment[463]. In stable asthma, the availability of reliable biomarkers and the understanding of different disease phenotypes and endotypes has revolutionised the management algorithms towards a precision, treatable traits approach[148]. On contrast, the utility of such an approach in the management of acute asthma is hindered by poor understanding of the underlying attack phenotypes and the lack of reliable, easily obtained biomarkers to allow precise treatment selection. As a result, the one-size-fits-all treatment approach with OCS remains the mainstay treatment option for AAs. Diagnosis and assessment of AAs are based on presenting symptoms and level of lung function impairment assessed using spirometry or peak flow[151].

Histological examination of lung tissue obtained using bronchial biopsies is the most accurate method of assessing airway inflammation, however, this method is invasive

and not suitable for routine clinical practice. The best alternative to lung bronchoscopy is induced sputum. Analysis of sputum differential cell count is widely used in research and is a well-established method for the assessment of inflammation biomarkers[464,465] and has shown to be useful in guiding treatment decisions[428,466]. However, sputum induction is invasive, time consuming, and has potential risks, particularly during acute deterioration of asthma.

Blood eosinophil count and FeNO are widely used for the treatment and management of T2-high asthma patients as proxy for eosinophilic activity[145], but their use in the context of AAs is limited and not well understood. BEC is a marker of T2-high inflammation and has been shown to correlate well with the level of asthma control[467], risk for AAs[467,468], and increased risk for re-admission after an asthma related hospitalisation[469]. Similarly, FeNO can also serve as a predictor of corticosteroid responsiveness[470] and an increased risk for AAs[471,472]. Given the different disease pathways each of these biomarkers represent, i.e, IL-5 linked more closely to maturation and systemic recruitment of blood eosinophils and FeNO more reflective of IL-13 mediated airway epithelial inflammation [473], their combination has shown to have a stronger prognostic and predictive value across different disease severities[474]. The role of BEC and FeNO in the management of AAs has recently been investigated by Couillard et al.[475] and Bafadhel et al.[430], but further knowledge regarding utility of these biomarkers in acute asthma is required before widespread use in clinical practice.

Overall thesis rationale and aims

Rationale

Despite the well-recognised heterogeneity of the underlying pathophysiological mechanisms underlying acute asthma, these episodes are uniformly treated with systemic corticosteroids based on non-asthma specific symptoms and subjective assessment of airflow limitation using peak expiratory flow despite the wide availability of point of care, feasible measures of airway inflammation and objective measures of airflow deterioration. While such tools are routinely employed in the management of chronic asthma, they remain underutilised in the acute setting, a gap largely attributable to the limited understanding of acute asthma pathophysiology and the

absence of a pathophysiology-based definition of acute exacerbations. These limitations have important clinical implications, including the risk of misdiagnosing asthma-mimicking conditions as acute asthma and the potential overuse of systemic corticosteroids, even in cases of objectively confirmed acute asthma. This thesis aims to address these critical gaps by highlighting the limitations of current acute asthma management guidelines, which often overlook the underlying disease biology, and by exploring the potential utility of point-of-care assessments of lung function and inflammation in guiding the management of acute asthma.

Aims

1. To provide a description of patients admitted to hospital with suspected asthma attacks with emphasis on recorded evidence of airflow limitation suggestive of acute asthma and clinical characteristic and demographic data of patients identified based on available electronic healthcare record data (Chapter 2). This chapter focuses on highlighting potential limitations of current management guidelines and identifying admissions misdiagnosed as acute asthma, which has not been thoroughly investigated previously.
2. To provide a prospective characterisation of asthma attack admissions with a focus on evidence of airflow limitation and airway inflammation assessed using objective point of care measures of lung function and inflammation (Chapter 3). The focus of this chapter is to allow the assessment of the potential utility of point of care measures of lung function and inflammation in the management of acute asthma and to allow an understanding of the pathophysiological mechanisms involved during acute episodes of asthma, which few studies have previously addressed.
3. To provide a detailed description of admissions of patients admitted with acute asthma and those with potentially asthma mimicking conditions and to highlight the potential utility of objective measures of lung function and inflammation in distinguishing between those patients (Chapter 4).
4. To provide a description of the clinical and biological response during recovery following hospital admission and treatment with systemic corticosteroids for acute asthma (Chapter 5). This chapter highlights the degree of lung function recovery in patients with and without airflow limitation at hospital admission and explores the

potential utility of inflammatory profiling of asthma attacks (at visit 1) in predicting lung function recovery (at visit 2).

Chapter 2: Audit of asthma attack admissions

Introduction

The definition of asthma attacks is vague and is often based on treatment received rather than objective evidence of symptoms worsening and lung function decline. In addition, the distinction between periods of poorly controlled asthma and genuine acute deterioration of asthma is difficult. As a result, such events are uniformly treated based on subjective assessment of symptoms and PEFR deterioration. Both the reported symptoms and clinical examination finding of 'wheeze' are not asthma specific[476], and PEFR, although objective, is effort dependent and requires maximal patient effort[477].

Moreover, many conditions mimic acute deterioration of asthma[403], and a group of "exacerbation-prone" patients with significant *presumed* asthma-related healthcare utilisation and frequent hospital admissions has been recognised[436]. Although some characteristics of these patients have been described, details regarding their AAs and AA admissions are lacking. Additionally, while suboptimal compliance with asthma attack management guidelines has been reported[478], the relationship between such non-compliance and patients' clinical or demographic characteristics has not been thoroughly explored. This study aimed to describe "real-life" hospitalised AA events from available electronic healthcare record data with an emphasis on objective evidence of airflow obstruction and patients with multiple AA admissions.

Aim

To describe AA admissions over a 1-year period with an emphasis on evidence of airflow obstruction and frequently admitted patients.

Primary objectives

- To describe the demographics, frequency of admission, and clinical characteristics of adult patients admitted to a secondary respiratory centre with a suspected asthma attack over a 1-year period prior to the pandemic.
- To determine whether these admissions are due to "true" asthma attacks, and other factors contributing to or responsible for AA admissions.

Secondary objectives

- Determine variations in systemic corticosteroid and antibiotic prescription for the treatment of acute asthma.
- Determine proportion who received the six elements of good practice for patients with asthma following asthma attacks.

Hypothesis

A proportion of patients admitted for AAs do not have evidence of acute airflow limitation suggestive of acute asthma.

Methodology

Study design

This is a retrospective audit of medical records.

Ethical approval

No ethical approval was required for this study which was part of a clinical audit approved by and registered with the Nottingham University Hospital (NUH), Nottingham City Hospital audit team (audit registration number 20-701C).

Eligibility criteria

Medical records of asthma attack admissions were audited based on the following eligibility criteria

Inclusion criteria

- Male or female aged ≥ 18 years.
- Admission to Nottingham City Hospital Respiratory Admissions Unit between 1st of January 2019 and 31st of December 2019.
- Primary admission diagnosis of an exacerbation of asthma.

Exclusion criteria

- Admission to other medical units, i.e. General medicine.
- A&E only admissions.

Study setting

The medical records of patients aged 18 or above admitted to A&E or inpatient wards at Nottingham City Hospital with coded primary diagnoses of asthma exacerbation or alternate diagnoses known to mimic asthma exacerbations during the period from the 1st of January 2019 to 31st of December 2019 were requested from the clinical audit team. This period was deliberately chosen as it was pre-pandemic. The International Classification of Disease 10 (ICD-10) codes pertaining to the following primary diagnoses were used to generate a list of single and consecutive admissions of patients hospitalised during the specified period: *asthma, asthma exacerbation, vocal cord dysfunction, dysfunctional breathing, and hyperventilation.* These codes were applied based on hospital discharge summaries, and were primarily used to ensure that the audited admissions reflected final clinical diagnoses made at discharge, rather than provisional labels assigned at triage or during initial assessment. Electronic health care records of admissions available on NUH Electronic Health Record software were manually accessed and audited. Audited admissions include those of single-time attenders (patients with one admission only during the audited period) admitted from the 1st of January until the 18th of July 2019 (182 patients; 182 admissions), and consecutive admissions of multiple-time attenders (patients with multiple admissions during the audited period) admitted from the 1st of January until the 19th of December 2019 (38 patients; 129 admissions). We deliberately only audited asthma attack visits admitted to the respiratory admissions unit to ensure admissions included were reviewed by a specialist respiratory clinician. Data were manually entered into a secure clinical cloud database (REDCap cloud v1.6) designed and managed by the Nottingham Respiratory Research Team.

Outcome measures

Primary endpoints

- The demographics, frequency of admission, and clinical characteristics of patients identified.
- The number and characteristics of frequent attendees and repeat admissions.
- The proportion of "Likely AA" admissions based on documented evidence of deterioration of asthma (Wheeze or PEFR limitation (≤75%) or both), "Unclear AA"

admissions (No wheeze/clear breath sound and no documented PEFR), and "Unlikely AA" admissions (No wheeze/clear breath sound and documented PEFR of >75%).

- A description of the characteristics of and factors involved in AA admissions in terms of severity, documented physiological symptoms and reported triggers, clinical investigations (i.e. differential cell counts and measures of infection), and contribution of other pathology (i.e. chest infection or asthma mimics).

Secondary endpoints

- Determine variations in prescription of systemic corticosteroid courses and antibiotics for asthma attacks.
- Determine proportion who received the six elements of good practice care following asthma attacks as specified in the National Asthma and Chronic Obstructive Pulmonary Disease Audit Programme (NACAP): Adult asthma clinical audit 2018/19[479]:
  - Inhaler technique check.
  - Maintenance medication review.
  - Adherence review.
  - Personalised asthma action plan issue/review.
  - Smoking cessation.
  - Post discharge follow-up.

Data collection

Data were extracted on three main areas: patient demographics, medical history and comorbidities, and asthma attack admission details.

Patient demographics

Demographic data collected include age, gender, and ethnicity. Postcodes were also collected to obtain Townsend socioeconomic scores. The Townsend Deprivation Index is a commonly used measure of material deprivation. Patients' postcodes were not stored in the study database.

Medical history and comorbidities

This included the source of previously diagnosed asthma, documented asthma severity and phenotype, and documented objective evidence of asthma. Data were collected from documents in the patients' medical records prior to the date of the asthma attack admission audited.

Objective evidence of asthma included FEV_1/FVC <0.7, spirometry with significant post-bronchodilator reversibility, positive methacholine challenge test, peak flow variability, blood eosinophils ≥0.4 $x10^9$/L, and FENO ≥25 ppb. Asthma treatment and appointments attended and missed in the previous 2 years were collected. Previously diagnosed airway diseases and other significant comorbidities were also recorded.

In cases where data are recorded but the source of the data was not documented, this was recorded as "unclear" source of data. Likewise, in cases where data are not documented this was recorded as "undocumented."

Asthma attack admission details

Documented evidence suggestive of acute asthma were recorded and include either or both of the following:

- Documented presence of wheeze on respiratory examination[2] at any point during admission.
- Documented PEFR of ≤75% of best PEFR[184] if documented, or calculated predicted PEFR if best PEFR not documented, at any point during admission.

The presence of clinical signs/physiological measurements associated with severity of asthma attacks, as per the British Thoracic Society (BTS) levels of severity of acute asthma in adults[184], were recorded; these include:

- Respiratory rate ≥25/min
- Heart rate ≥110/min
- SpO2 <92%
- PaO2 <8 kPa
- Normal PaCO2 on an arterial blood gas
- Increased PaCO2 on an arterial blood gas
- Exhaustion

- Hypotension
- Drowsiness
- Silent chest

Computed tomography (CT) scan and chest radiograph (CXR) findings, microbiology, and C-reactive protein (CRP) results were collected for findings suggestive of involvement of other pathology. For the purpose of inflammatory phenotyping, blood eosinophil results from each admission were collected. All acute asthma treatment received during admissions were recorded. Documented secondary diagnoses, reported triggers, and six elements of good practice care for patients with asthma received during admission were also recorded.

Data management and analysis

Data were retrospectively collected and stored in a secured web-based server (REDCap, Research Electronic Data Capture)[480]. Microsoft Excel was used to prepare and clean the data. Statistical analyses were performed using R software, version 2024.04.2+764 (R Foundation for Statistical Computing, Vienna, Austria).

Missing data

Missing data were present across several variables, particularly for peak expiratory flow rate at admission and at discharge. These data were not available in all admissions, largely due to incomplete clinical documentation in the electronic health records and the retrospective nature of the audit. Given the clinical setting and audit scope, it was not possible to retrieve missing values or confirm whether tests were performed but not documented. No data imputation techniques were applied. Analyses were conducted on an available data, with each variable or comparison based on the number of available observations. Where appropriate, the denominator was specified in tables to indicate the number of observations included in each analysis.

Statistical analysis

Data were descriptively expressed using mean ± SD for normally distributed data, median (IQR) for skewed distribution, and numbers and percentages for categorical data. Continuous, normally distributed variables were compared using two-samples t-

test. For skewed variables, a Wilcoxon signed-rank test was performed. Distribution of data was visually assessed. Categorical data were compared using Chi-squared test or Fisher's exact where expected values are below 5. Number of observations presented will vary depending on available data.

To determine whether there is a trend towards an increase in the number of admissions with no objective evidence of acute asthma with increasing frequency of admissions, a Chi-squared test for trend was performed.

A univariate logistic model was used to identify factors that are potentially associated with no obvious obstruction admissions. These include age, gender, asthma mimics, pre-admission diagnosis of asthma, higher treatment steps (4 or 5), Townsend score, and number of admissions. Using a P value of <0.05, factors significantly associated with the outcome were included in the multiple regression model.

Results

Medical records identified using ICD-10 codes

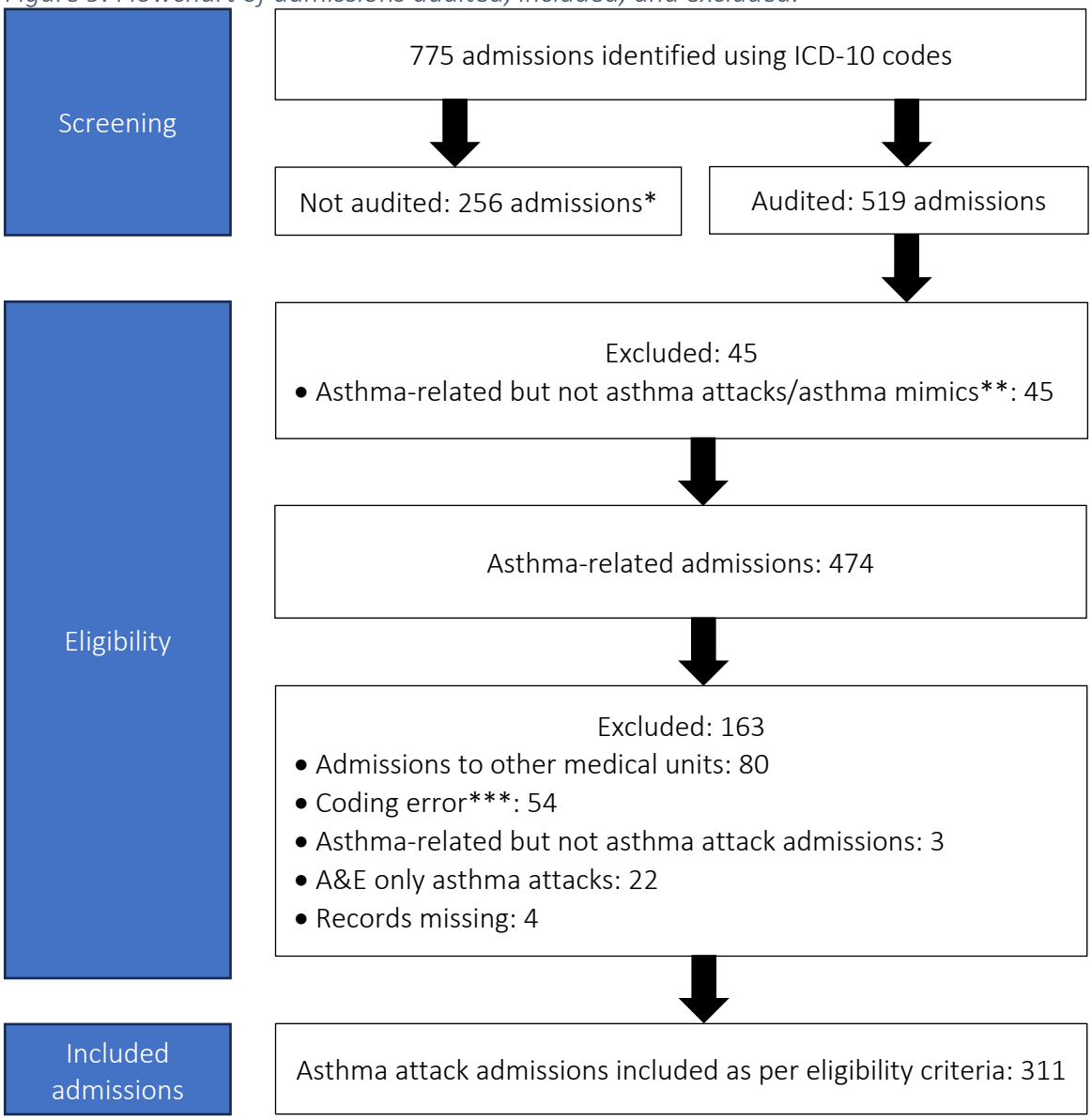
The ICD-10 code search returned 775 admissions, 730 of which were asthma-related (Table 2). Electronic health records of 519 asthma-related admissions were accessed and audited, of which 311 met the audit's eligibility criteria; the remaining admissions (n= 256) were not audited due to a sufficient number of admissions included at 311 admissions (Figure 9).

Table 2. Admissions identified using the ICD-10 codes.

ICD-10 code	Number of admissions
• *Asthma-related**	730
• *Hyperventilation*	6
• *Other and unspecified abnormalities of breathing*	29
• *Hiccough*	7
• *Other diseases of vocal cords*	1
• *Stridor*	2
• *Total*	775

**Asthma unspecified, status asthmaticus, predominantly allergic asthma.*

Figure 9. Flowchart of admissions audited, included, and excluded.



**A sufficient number was reached, and the remaining admissions were not audited. **Asthma-related diagnoses/asthma mimics include Hyperventilation, Other and unspecified abnormalities of breathing, Hiccough, Other diseases of vocal cords, Stridor. ***Coded as asthma attacks using ICD-10 codes, but actual discharge diagnosis was different, i.e., Pleural effusion, Abdominal pain, Possible PE, etc.*

Patients identified

A total of 220 patients were identified. The majority of the patients had only one AA admission (single-time attendees, STA) during the audited period, 38 (17.3%) had multiple admissions (>1 AA admission, multiple-time attendees, MTA), and 7 (3.2%) had ≥4 AA admissions (frequent-time attendees, FTA) (Table 3). A summary of missing data is presented in Appendix A.

Table 3. Patients identified and frequency of admissions.

	All patients	1 admission	>1 admission	2 admissions	3 admissions	≥4 admissions
	N (%) unless stated otherwise					
Number of patients	220	182 (82.7)	38 (17.3)	21 (9.5)	10 (4.5)	7 (3.2)
Number of admissions	311	182 (58.5)	129 (41.5)	42 (13.5)	30 (9.6)	57 (18.3)
Patient to visit ratio	1:1.4	1:1	1:3.4	1:2	1:3	1:8.1

Single vs. multiple-time attendees (>1 admission)

Demographics and medical history

The demographic features of patients identified, categorised according to the frequency of attendance, are shown in Table 4. The mean age of all patients identified was 50.5 years; MTA were more likely to be younger compared to STA (44.5 vs. 51.8, P= 0.008). Most patients were female (76.4%) and White or White British (74.6%), with a higher proportion of female patients observed in MTA compared to STA (89.5% vs. 73.6%, P= 0.04). The mean Townsend socioeconomic score was 0.96 and was higher in MTA compared to STA (P= 0.06). The median length of hospital stay (LOS) was 2.7 days; LOS was increased in MTA compared to STA (P= 0.02). There was a borderline significant, weak positive linear correlation between the number of admissions per patient and Townsend scores (rho: 0.12, P= 0.06).

Table 4. Demographic characteristics according to admission frequency.

Demographics	All patients (N= 220)	STA (n= 182)	MTA (n= 38)	P value
	N (%) unless stated otherwise			
Age (Mean ± SD) (years)	50.5 ± 19.0	51.8 ± 19.7	44.5 ± 14.1	0.008
Gender				
• *Male*	52 (23.6)	48 (26.4)	4 (10.5)	0.04
• *Female*	168 (76.4)	134 (73.6)	34 (89.5)	
Ethnicity				
• *White/White British*	164 (74.6)	134 (73.6)	30 (78.9)	0.16
• *Black/Black British*	5 (2.3)	4 (2.2)	1 (2.6)	
• *Asian/Asian British*	18 (8.2)	13 (7.1)	5 (13.2)	
• *Not Known*	33 (15.0)	31 (17.0)	2 (5.3)	
Townsend score (Mean ± SD)	0.96 ± 3.16	0.78 ± 3.18	1.82 ± 2.97	0.06
Number of admissions Median (IQR)	1 (1 - 1)	1 (1 - 1)	2 (2 - 3)	
Length of stay in days Median (IQR)	2.7 (1 - 4)	2 (1 - 4)	3 (2 - 6)	0.02

The majority of the patients identified had previously been diagnosed with asthma prior to their attack admission in 2019 (the audited period), with GP being the source of the diagnosis in the majority of the patients. Moreover, 67 (31.8%) of the patients received asthma specialist care at NUH before 2019 and hence had an asthma specialist diagnosis (Table 5). Compared to STA, MTA were more likely to be under asthma specialist care and to have an asthma specialist diagnosis (27.6% vs. 51.4%, P= 0.02). The number of previously diagnosed comorbidities and asthma mimics was higher in MTA compared to STA (P= 0.07 and P= 0.003, respectively). This includes rhinitis (P= 0.005), depression/anxiety (P <0.001), Inducible laryngeal obstruction (ILO) (P= 0.005), and Breathing Pattern Disorder (BPD) (P= 0.09) (Table 5). Moreover, the proportion of patients with asthma-related intensive treatment unit (ITU) admission during the period of the audit was also higher in MTA compared to STA (10.5% vs. 0.6%, P= 0.003).

Table 5. Medical history and comorbidities according to admission frequency.

Medical history and comorbidities	All patients (N= 220)	STA (n= 182)	MTA (n= 38)	P value
	N (%)			
Previously diagnosed asthma	211 (95.9)	174 (95.6)	37 (97.4)	1
Source of diagnosis				
• *GP*	137 (64.9)	120 (68.9)	17 (45.9)	**0.02**
• *Asthma Specialist*	67 (31.8)	48 (27.6)	19 (51.4)	
• *Unclear*	7 (3.3)	6 (3.5)	1 (2.7)	
Significant comorbidities	166 (75.5)	133 (73.1)	33 (86.8)	0.07
• *Gastroesophageal Reflux Disease (GERD)*	25 (11.4)	19 (10.4)	6 (15.8)	0.39
• *Rhinitis*	73 (33.2)	53 (29.1)	20 (52.6)	**0.005**
• *Upper airway cough syndrome*	2 (0.91)	2 (1.1)	0 (0)	1
• *Nasal polyps*	7 (3.2)	6 (3.3)	1 (2.6)	1
• *Depression/Anxiety*	53 (24.1)	35 (19.2)	18 (47.4)	**<0.001**
• *Obesity (BMI ≥30)*	111 (50.5)	89 (48.9)	22 (57.9)	0.31
Airway diseases	37 (16.8)	28 (15.4)	9 (23.7)	0.21
• *COPD*	18 (8.2)	16 (8.8)	2 (5.3)	0.75
• *Bronchiectasis*	12 (5.5)	9 (4.9)	3 (7.9)	0.44
• *Obstructive sleep apnoea*	12 (5.5)	8 (4.4)	4 (10.5)	0.13
Asthma Mimics	17 (7.7)	9 (4.9)	8 (21.1)	**0.003**
• *Inducible laryngeal obstruction (ILO)*	8 (3.6)	3 (1.7)	5 (13.2)	**0.005**
• *Breathing Pattern Disorder*	11 (5.0)	7 (3.9)	4 (10.5)	0.09
• *Excessive dynamic airway collapse*	1 (0.5)	0 (0)	1 (2.6)	0.17
Smoking status				
• *Current smoker*	36 (16.4)	33 (18.1)	3 (7.9)	0.22
• *Non-smoker*	174 (79.1)	140 (76.9)	34 (89.5)	
• *Not documented*	10 (4.6)	9 (4.9)	1 (2.6)	
Asthma related ITU admission*	5 (2.3)	1 (0.6)	4 (10.5)	**0.003**

**During the audited period.*

Previous asthma history

Of those under the care of an asthma specialist at NUH, 38.8% had severe, difficult, or brittle asthma, and 44.8% had eosinophilic asthma. Moreover, 34.6% of patients had documented evidence of asthma, including evidence of airflow obstruction (12.3%) and T2-high inflammation (27.7%). Previously documented objective evidence of asthma

was higher in MTA compared to STA (P <0.001), including evidence of airflow obstruction (P= 0.09) and increased T2-high inflammation (P= 0.01) (Table 6). MTA were more likely to have had asthma appointments (44.7% vs. 20.9%, P= 0.002) and to have missed asthma appointments (34.2% vs. 12.6%, P= 0.001) in the two years preceding the asthma attack admission audited compared to STA (Table 6).

Table 6. Previously documented asthma history according to admission frequency.

Previously diagnosed asthma	All patients (N= 211)	STA (n=174)	MTA (n=37)	P value
Asthma-specific history	N (%)			
Asthma severity* (n=67)				
• *Mild/Moderate*	5 of 67 (7.5)	1 of 48 (2.1)	4 of 19 (21.1)	0.003
• *Severe/ Difficult/ Brittle*	26 of 67 (38.8)	16 of 48 (33.3)	10 of 19 (52.6)	
• *Not documented*	36 of 67 (53.7)	31 of 48 (64.6)	5 of 19 (26.3)	
Asthma phenotype* (n=67)				
• *Eosinophilic*	30 of 67 (44.8)	22 of 48 (45.8)	8 of 19 (42.1)	0.83
• *Not documented*	37 of 67 (55.2)	26 of 48 (54.2)	11 of 19 (57.9)	
Objective evidence of asthma**	76 (34.6)	54 (29.7)	22 (57.9)	<0.001
• *Airflow obstruction*	27 (12.3)	19 (10.4)	8 (21.1)	0.09
• *T2-high inflammation*	61 (27.7)	44 (24.2)	17 (44.7)	0.01
Attended asthma appointments (last 2 years)***	55 (25.0)	38 (20.9)	17 (44.7)	0.002
Asthma appointments missed (last 2 years)***	36 (16.4)	23 (12.6)	13 (34.2)	0.001

**In those under an asthma specialist. **Where documented in medical notes (in those under an asthma specialist) or lab results prior to the attack admission audited: FEV_1/FVC <0.7, Spirometry with reversibility, positive methacholine challenge, PEFR variability, Blood eosinophils (≥.0.4), or Raised FeNO (≥25 ppb). ***Respiratory physician appointment.*

Asthma treatment

The majority of the patients were on asthma treatment at the time of the audited admissions; however, MTA were more likely to have been on GINA step 5 treatment compared to STA (32.4% vs. 8.4%, P= 0.002) (Table 7). Multiple-time attendees were also more likely to be on biological treatment for asthma than STA.

Table 7. Asthma treatment according to admission frequency.

Previously diagnosed asthma	All patients (N= 211)	STA (n=174)	MTA (n=37)	P value
Asthma treatment	N (%)			
On GINA treatment Step	188 (85.5)	154 (84.6)	34 (89.5)	0.44
• *Step 1*	0 of 188 (0)	0 of 154 (0)	0 of 34 (0)	
• *Step 2*	7 of 188 (3.7)	7 of 154 (4.6)	0 of 34 (0)	
• *Step 3*	107 of 188 (56.9)	92 of 154 (59.7)	15 of 34 (44.1)	0.002
• *Step 4*	50 of 188 (26.6)	42 of 154 (27.3)	8 of 34 (23.5)	
• *Step 5*	24 of 188 (12.8)	13 of 154 (8.4)	11 of 34 (32.4)	
On Biological treatment	13 (5.9)	6 (3.3)	7 (18.4)	0.002

Single vs. frequent-time attendees (≥4 admissions)

Demographics and medical history

Similar to MTA, age was significantly lower in FTA compared to STA (P= 0.03). Moreover, Townsend score and LOS were higher in FTA compared to STA (P= 0.02 and P= 0.06, respectively) (Table 8). The correlation between the number of admissions per patient and Townsend scores in FTA was not significant (rho: 0.16, P= 0.74). A summary of missing data is presented in Appendix A.

Table 8. Demographic characteristics of single-time attendees compared to frequent-time attendees.

Demographics	STA (n= 182)	FTA (n= 7)	P value
	N (%) unless stated otherwise		
Age Median (IQR) (years)	53.0 (34.3 - 68.0)	34.0 (26.5 - 45.5)	**0.03**
Gender			
• *Male*	48 (26.4)	1 (14.3)	0.67
• *Female*	134 (73.6)	6 (85.7)	
Ethnicity			
• *White/White British*	134 (73.6)	5 (71.4)	0.19
• *Black/Black British*	4 (2.2)	0 (0)	
• *Asian/Asian British*	13 (7.1)	2 (28.6)	
• *Not Known*	31 (17.0)	0 (0)	
Townsend score (Mean ± SD)	0.78 ± 3.18	3.3 ± 2.1	**0.02**
Number of admissions Median (IQR)	1 (1 - 1)	4 (4 - 6.5)	
Length of stay in days Median (IQR)	2 (1 - 4)	4.5 (3.2 - 5.4)	0.06

The majority of the patients from both groups had previously been diagnosed with asthma. Similar to MTA, FTA were more likely to be under asthma specialist care and to have an asthma specialist diagnosis compared to STA (P= 0.006). FTA were more likely to have a diagnosis of rhinitis (P= 0.03), depression/anxiety (P= 0.03), obstructive sleep apnoea (P= 0.04), and asthma mimics (P <0.001), including ILO (P <0.001), and BPD (P= 0.04). No difference was observed in smoking status; however, the proportion of patients who had asthma-related ITU admissions during the audited period tended to be higher in the FTA compared to STA (14.3% vs. 0.6%, P= 0.07) (Table 9).

Table 9. Medical history and comorbidities in single-time attendees compared to frequent-time attendees.

Medical history and comorbidities	STA (n= 182)	FTA (n= 7)	P value
	N (%)		
Previously diagnosed asthma	174 (95.6)	7 (100)	1
Source of diagnosis			
GP	120 (65.9)	1 (14.3)	0.006
Asthma Specialist	48 (26.4)	6 (85.7)	
Unclear	14 (7.7)	0 (0)	
Significant comorbidities	133 (73.1)	7 (100)	0.19
• *Gastroesophageal Reflux Disease (GERD)*	19 (10.4)	2 (28.6)	0.17
• *Rhinitis*	53 (29.1)	5 (71.4)	0.03
• *Upper airway cough syndrome*	2 (1.1)	0 (0)	1
• *Nasal polyps*	6 (3.3)	1 (14.3)	0.23
• *Depression/Anxiety*	35 (19.2)	4 (57.1)	0.03
• *Obesity (BMI ≥30)*	89 (48.9)	4 (57.1)	0.72
Airway diseases	28 (15.4)	2 (28.6)	0.3
• COPD	16 (8.8)	0 (0)	1
• Bronchiectasis	9 (4.9)	1 (14.3)	0.32
• Obstructive sleep apnoea	8 (4.4)	2 (28.6)	0.04
Asthma Mimics	9 (4.9)	4 (57.1)	<0.001
• *Inducible laryngeal obstruction (ILO)*	3 (1.7)	3 (42.9)	<0.001
• *Breathing Pattern Disorder (BPD)*	7 (3.9)	2 (28.6)	0.04
• *Excessive dynamic airway collapse*	0 (0)	0 (0)	
Smoking status			
• Current smoker	33 (18.1)	1 (14.3)	0.55
• Non-smoker	140 (76.9)	5 (71.4)	
• Not documented	9 (4.9)	1 (14.3)	
Asthma related ITU admission*	1 (0.6)	1 (14.3)	0.07

**During the audited period.*

Previous asthma history

Frequent-time attendees were more likely to have previously documented evidence of airflow obstruction (P= 0.04) (Table 10). The proportion of asthma appointments attended and missed in the two years prior to the audited admission were significantly increased in the FTA compared to the STA (Table 10).

Table 10. Previously documented asthma history in single-time attendees compared to frequent-time attendees.

Previously diagnosed asthma	STA (n=174)	FTA (n=7)	P value
Asthma-specific history	N (%)		
Asthma severity* (n= 54)			
• *Mild/Moderate*	1 of 48 (2.1)	2 of 6 (33.3)	0.02
• *Severe/ Difficult/ Brittle*	16 of 48 (33.3)	2 of 6 (33.3)	
• *Not documented*	31 of 48 (64.6)	2 of 6 (33.3)	
Asthma phenotype* (n=54)			
• *Eosinophilic*	22 of 48 (45.8)	1 of 6 (16.7)	0.22
• *Not documented*	26 of 48 (54.2)	5 of 6 (83.3)	
Objective evidence of asthma**	54 (29.7)	4 (57.1)	0.2
• *Airflow obstruction*	19 (10.4)	3 (42.9)	0.04
• *T2-high inflammation*	44 (24.2)	3 (42.9)	0.37
Attended asthma appointments (last 2 years)***	38 (20.9)	5 (71.4)	0.007
Asthma appointments missed (last 2 years)***	23 (12.6)	4 (57.1)	0.009

**In those under an asthma specialist. **Where documented in medical notes (in those under an asthma specialist) or lab results prior to the attack admission audited: FEV_1/FVC <0.7, Spirometry with reversibility, positive methacholine challenge, PEFR variability, Blood eosinophils (≥.0.4), or Raised FeNO (≥25 ppb). ***Respiratory physician appointment.*

Asthma treatment

The proportion of patients on GINA step 5 treatment step was significantly higher in FTA compared to STA (Table 11). The proportion of patients on biological treatment for asthma was significantly higher in FTA.

Table 11. Asthma treatment in single-time attendees compared to frequent-time attendees.

Previously diagnosed asthma	STA (n=174)	FTA (n=7)	P value
Asthma treatment	N (%)		
On GINA treatment Step	154 (88.5)	7 of 7 (100)	0.59
• *Step 1*	0 of 154 (0)	0 of 7 (0)	0.009
• *Step 2*	7 of 154 (4.6)	0 of 7 (0)	
• *Step 3*	92 of 154 (59.7)	2 of 7 (28.6)	
• *Step 4*	42 of 154 (27.3)	1 of 7 (14.3)	
• *Step 5*	13 of 154 (8.4)	4 of 7 (57.1)	
On Biological treatment	6 (3.5)	3 (42.9)	0.002

Asthma attack admissions audited

In total, 311 AA admissions were identified and audited.

Objective evidence of airflow obstruction

Documented breath sounds

In the 311 AA admissions audited, breath sounds were documented as expiratory wheeze in 106 (34.1%) and unspecified wheeze (e.g., wheeze or wheeze throughout) in 105 (33.8%) (Table 12). Both expiratory and unspecified wheeze were considered objective evidence of an AA and will be referred to as *Wheeze+*. Moreover, variable breath sounds were documented in 13 (4.2%) AA admissions. These include non-asthma-related breath sounds, i.e., crackles or instances where it was challenging to determine the specific breath sound documented; for example, two completely different breath sounds, stridor and rhonchi, were documented. Clear chest or no wheeze were documented in 87 (27.9%) AA admissions (Table 12); clear, no wheeze, and variable breath sounds will be referred to as *No Wheeze*. A summary of missing data is presented in Appendix A.

Documented peak expiratory flow rate

Peak expiratory flow rate was documented in 262 (84.2%) AA admissions. Patients' best PEFR was documented in 231 (74.3%) AA admissions. 207 (66.6%) of the admissions had documented PEFR limitation (PEFR ≤75% of patient's documented best or calculated PEFR), and 55 (17.7%) admissions had no PEFR limitation (PEFR >75% of patient's documented best or calculated PEFR). PEFR was not documented in 49 (15.8%) AA admissions (Table 12).

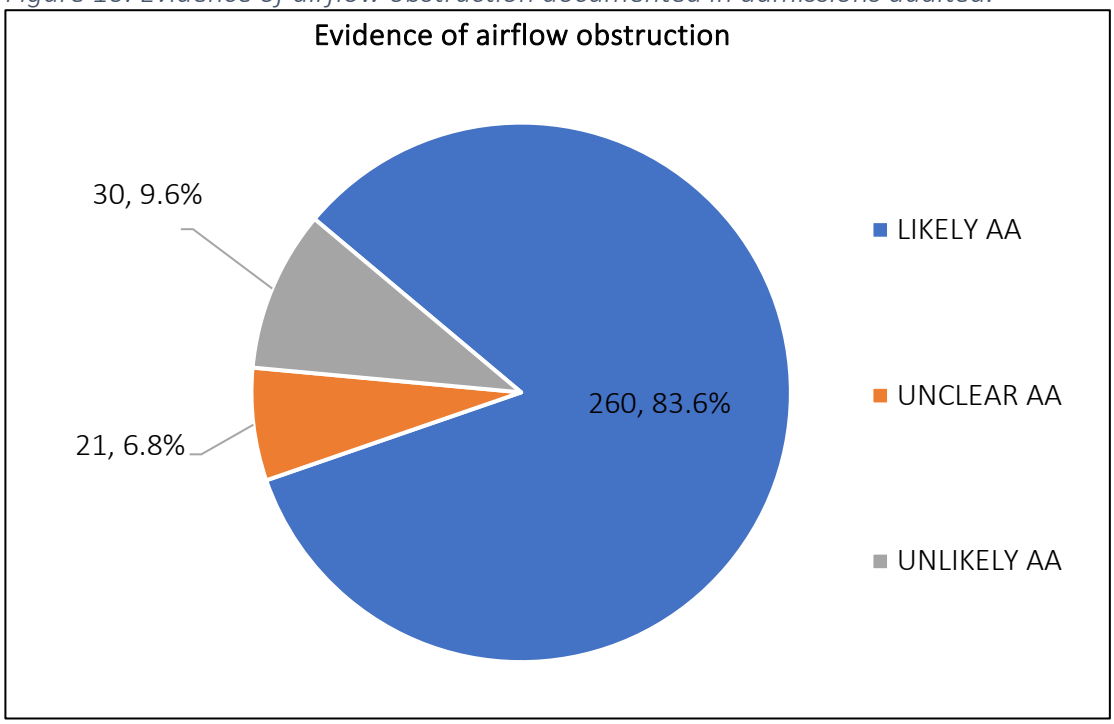
Table 12. Documented breath sounds and PEFR.

Objective evidence of airflow obstruction	AA admissions (N=311)
	N (%)
Breath sound documented	**311 (100)**
• *Expiratory wheeze*	106 (34.1)
• *Wheeze unspecified*	105 (33.8)
• *Variable/non-asthma related*	13 (4.2)
• *Clear/no wheeze*	87 (27.9)
PEFR documented	**262 (84.2)**
• *PEFR limitation (≤75%)*	207 (66.6)
• *PEFR >75%*	55 (17.7)
• *PEFR not performed/not documented*	49 (15.8)

Evidence of acute asthma

In 311 AA admissions, evidence of airflow obstruction (EAO), based on *Wheeze+* (expiratory wheeze or unspecified wheeze) and/or PEFR limitation (PEFR ≤75%), was documented in 260 (83.6%) AA admissions; those were considered to be *likely AAs* (Figure 10). 21 (6.8%) of the admissions were *unclear AAs,* as these had *No Wheeze* (clear, no wheeze, variable breath sounds), but no PEFR was documented (Figure 10). 30 (9.6%) of the admissions were labelled as *unlikely AAs* as there was documentation of *No Wheeze* and a PEFR of >75% of patients documented best or calculated PEFR. Both *unclear AAs* and *unlikely AAs*, 51 (16.4%), are considered events lacking sufficient objective evidence suggestive of acute asthma and are referred to as *no obvious obstruction* (NOO) events.

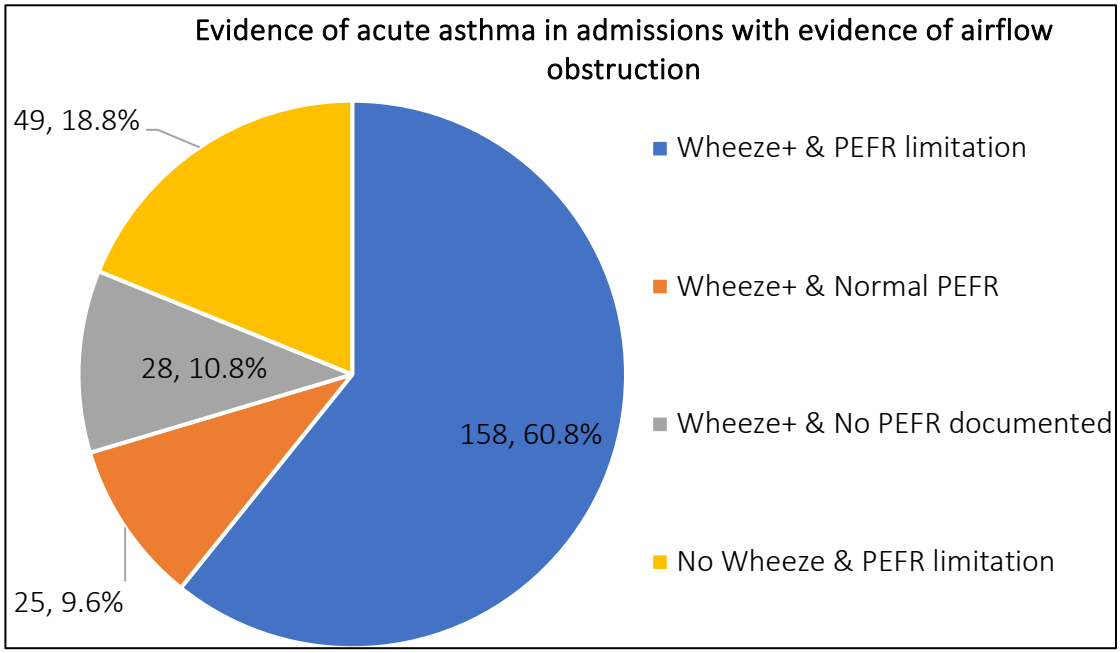
Figure 10. Evidence of airflow obstruction documented in admissions audited.



Evidence of acute asthma in admissions with evidence of airflow obstruction (n= 260)

In 260 AA admissions with evidence of airflow obstruction, the diagnoses of AA were based on wheeze+, along with PEFR limitation in 158 (60.8%). Wheeze+ and PEFR above 75% were documented in 25 (9.6%) AA admissions. Wheeze+ and undocumented PEFR was the case in 28 (10.8%) admissions, and PEFR limitation was the sole objective evidence of an AA in 49 (18.8%) admissions (Figure 11).

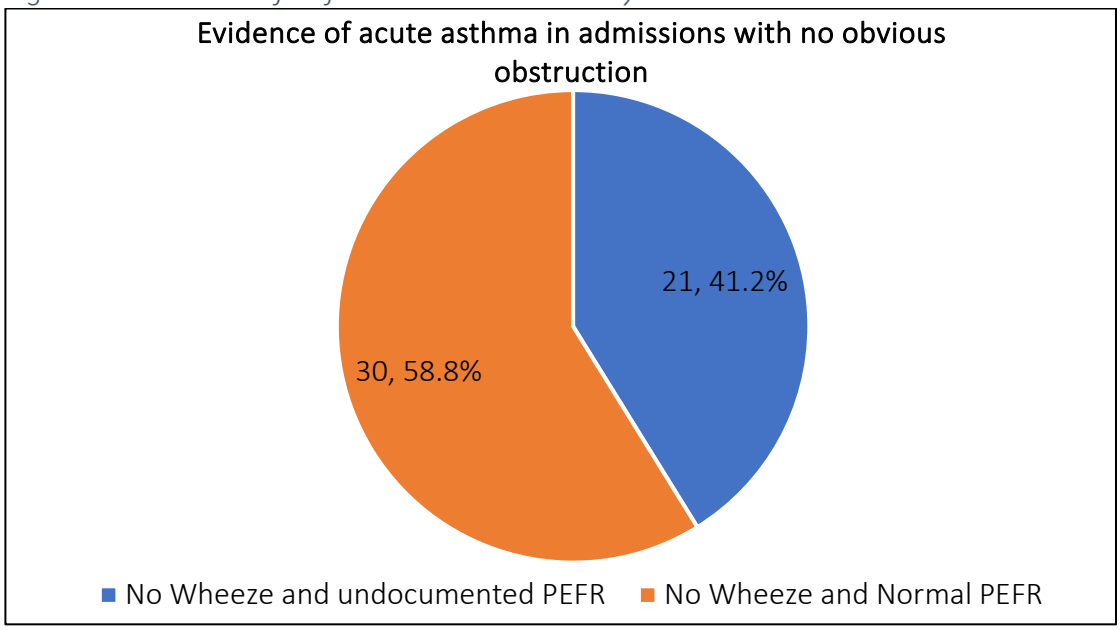
Figure 11. Evidence of airflow obstruction documented in likely asthma attack admissions.



Features of acute asthma in admissions with no obvious obstruction (n= 51)

Of the NOO admissions, 21 (41.2%) had *No Wheeze* and undocumented PEFR, while 30 (58.8%) had *No Wheeze* and PEFR above 75% (Figure 12).

Figure 12. Evidence of airflow obstruction in likely not asthma attack admissions.



Characteristics of admissions with evidence of airflow obstruction (n= 260) vs. no obvious obstruction admissions (n= 51)

Clinical characteristics

As expected, as PEFR is one of the measures used to categorise admissions with and without EAO, median PEFR% was significantly increased in admissions with NOO (P <0.001). The median length of hospital stay was significantly higher in AAs with EAO compared to those without (3 days vs. 2 days, P= 0.02). The proportion of patients with no signs or features of acute asthma, as per the BTS guidelines for acute asthma severity, was significantly higher in the NOO admissions (35.3% vs. 6.2%, P<0.001). There was no difference in the number of ITU admissions or radiology findings between the groups (Table 13).

The proportion of secondary diagnoses contributing to the admission was significantly increased in the admissions with NOO (P= 0.004). These include exacerbation of other airway diseases (P= 0.04), lower respiratory tract infection (P= 0.009), and BPD (P= 0.03).

The proportion of patients with blood eosinophil count ≥0.4 x10^^{9}/L was significantly higher in the AAs with EAO (P= 0.04). Although the median BEC was significantly lower in those on systemic anti-inflammatory treatment (low-dose maintenance OCS, biologics, or pre-admission treatment with OCS) at the time of hospital admission (0 x10^^{9}/L vs. 0.10 x10^^{9}/L, P <0.001), the proportion of patients on systemic anti-inflammatory treatment was not different between the groups (P= 0.85) (Table 14) and excluding those on systemic anti-inflammatory treatment, the proportion of those with increased BEC was still higher in the admissions with EAO (P= 0.05). The number of patients with CRP ≥10 or positive viral or bacterial microbiology was not different between the groups (Table 13).

Table 13. Clinical characteristics in admission with vs. without evidence of airflow obstruction.

Clinical characteristics	All admissions (N= 311)	AAs with EAO (n=260)	AAs with NOO (n=51)	P value
	N (%) unless stated otherwise			
On admission PEFR Median (IQR) (n= 262)	51.4 (40.1 - 70.3)	50.0 (39.9 - 60.0)	83.3 (77.9 - 87.9)	**<0.001**
LOS Median (IQR)	3 (1 - 5)	3 (1 - 5)	2 (1 - 3)	**0.02**
Requiring ITU admission	11 (3.5)	8 (3.1)	3 (5.9)	0.39
Acute asthma severity*				
• *No signs of acute asthma*	34 (10.9)	16 (6.2)	18 (35.3)	**<0.001**
• *Moderate*	75 (24.1)	75 (28.9)	0 (0)	
• *Acute severe*	87 (27.9)	67 (25.8)	20 (39.2)	
• *Life-threatening*	84 (27.0)	75 (28.9)	9 (17.7)	
• *Near-fatal*	31 (9.9)	27 (10.4)	4 (7.8)	
Abnormal CT/CXR findings	12 (3.9)	10 (3.9)	2 (3.9)	1
Secondary diagnosis	126 (40.5)	96 (36.9)	30 (58.8)	**0.004**
• Exacerbation of other airway diseases**	9 (2.9)	5 (1.9)	4 (7.8)	**0.04**
• Pneumonia	15 (4.8)	13 (5.0)	2 (3.9)	1
• LRTI	82 (26.4)	61 (23.5)	21 (41.2)	**0.009**
• *ILO*	1 (0.3)	0 (0)	1 (1.9)	0.16
• BPD	5 (1.6)	2 (0.8)	3 (5.9)	**0.03**
• Anxiety/Depression	5 (1.61)	4 (1.5)	1 (1.9)	0.51
AA trigger identified	145 (46.6)	123 (47.3)	22 (43.1)	0.58
• Coryzal/flu-like symptoms	126 (40.5)	108 (41.5)	18 (35.3)	0.41
• Pollutants***	6 (1.9)	3 (1.2)	3 (5.9)	0.06
• Outdoor allergens****	6 (1.9)	6 (2.3)	0 (0)	0.59
• Cold air & changing weather	6 (1.93)	5 (1.92)	1 (1.96)	1
• Exercise	1 (0.32)	1 (0.38)	0 (0)	1
Symptoms duration prior to admission (in days) Median (IQR)	2.0 (1.0 - 5.5)	3.0 (1.0 - 6.6)	2.0 (1.0 - 4.8)	0.9
Inflammation and infection				
BEC ≥0.4 x10^^{9}/L	55 (17.7)	51 (19.6)	4 (7.8)	**0.04**
CRP ≥10 mg/L	117 (37.6)	94 (36.2)	23 (45.1)	0.2
Microbiology results				
Positive Viral microbiology†	55 of 151 (36.4)	50 of 129 (38.8)	5 of 22 (22.7)	0.15
Positive Bacterial microbiology‡	3 of 71 (4.2)	3 of 58 (5.2)	0 of 13 (0)	1

**As per the BTS guideline. **COPD/bronchiectasis. ***Smoke, aerosol fresheners, cleaning products, perfumes. ****Pollen/trees. †In those with viral PCR results available. ‡In those with sputum Mc&s results available. LOS: Length of hospital stay. LRTI: Lower Respiratory Tract Infection. ILO: Inducible laryngeal obstruction. BPD: Breathing Pattern Disorders. BEC: Blood eosinophil count. CRP: C-reactive protein.*

Treatment received

The proportion of patients on pre-admission systemic anti-inflammatory treatment (Low-dose maintenance OCS, biologics, or pre-admission treatment with OCS) was similar between the groups. There was no difference in the number of those who received severe acute asthma treatment (i.e., magnesium sulphate and theophylline); however, those with NOO events were less likely to have received systemic corticosteroids (92.2% vs. 99.2%, P= 0.008), possibly due to the involvement of secondary diagnoses contributing to these admissions. The number of admissions treated with antibiotics was not different between the groups (Table 14).

Table 14. Treatment received in admissions with vs. without evidence of airflow obstruction.

Treatment received	All admissions (N= 311)	AAs with EAO (n=260)	AAs with NOO (n=51)	P value
	N (%) unless stated otherwise			
Pre-admission systemic anti-inflammatory*	58 (18.65)	48 (18.46)	10 (19.61)	0.85
Magnesium sulphate	53 (17.0)	43 (16.5)	10 (19.6)	0.59
Theophylline	19 (6.1)	17 (6.5)	2 (3.9)	0.75
Antibiotic treated	126 (40.5)	108 (41.5)	18 (35.3)	0.4
SCS treated	305 (98.1)	258 (99.2)	47 (92.2)	**0.008**
SCS duration in days (Mean ± SD) (n= 305)	7.3 ± 1.9	7.3 ± 1.9	7.3 ± 2.1	0.9

**Low-dose maintenance OCS, biologics, or pre-admission treatment with OCS. SCS: systemic corticosteroids.*

Cumulative systemic corticosteroid exposure

A high cumulative SCS exposure was noted, especially in the MTA and FTA groups (Table 15).

Table 15. Systemic corticosteroid treatment and cumulative exposure according to admission frequency.

SCS exposure	All patients (N= 220)	STA (n= 182)	MTA (n= 38)	P value	STA (n= 182)	FTA (n= 7)	P value
	N (%) unless stated otherwise				N (%) unless stated otherwise		
SCS treated	218 (99.1)	180 (98.9)	38 (100)	1	180 (98.9)	7 (100)	1
Cumulative SCS (mg)* Median (IQR)	280 (280 - 400)	280 (280 - 280)	680 (560 - 935)	<0.001	280 (280 - 280)	1160 (1010 - 1660)	<0.001

**In patients treated with systemic corticosteroids.*

Discharge characteristics

On-discharge PEFR% was documented in 195 of the 262 AA admissions with on-admission PEFR documented. As expected, as NOO events had initially no EAO, on-discharge median PEFR% was higher in those with NOO (92.4% vs. 77.8%, P <0.001). Only 97 (53.6%) of the admissions with EAO had PEFR >75% on discharge (Table 16). The percentage of PEFR improvement was significantly higher in the admissions with EAO compared to those with NOO (P <0.001).

Only 79 (25.4%) of the admissions audited received the National Asthma and COPD Audit Programme six elements of good practice care before discharge. There was no difference in the proportion of post admission follow-up appointments arranged, attended, or missed between the groups (Table 16).

Table 16. Discharge characteristics of admissions with vs. without evidence of airflow obstruction.

Discharge characteristics	All admissions (N= 311)	AAs with EAO (n=260)	AAs with NOO (n=51)	P value
	N (%) unless stated otherwise			
On Discharge PEFR%* Median (IQR) (n= 195)	78.7 (63.5 - 89.7)	77.8 (62.7 - 88.9)	92.4 (83.7 - 88.9)	**<0.001**
PEFR >75% on discharge*	111 of 195 (56.9)	97 of 181 (53.6)	14 of 14 (100)	
PEFR% improvement* (Mean ± SD)	32.5 ± 16.5	34.0 ± 16.1	13.5 ± 7.0	**<0.001**
6 elements of good practice care**	79 (25.4)	71 (27.3)	8 (15.7)	0.08
Post admission Follow-up arranged	**189 (60.8)**	**162 (62.3)**	**27 (52.9)**	0.21
• Post admission Follow-up attended	142 (75.1)	120 (74.1)	22 (81.5)	0.40
• Post admission Follow-up missed	47 (24.9)	42 (25.9)	5 (18.5)	
Medication changes at discharge	92 (29.6)	81 (31.2)	11 (21.6)	0.19

**In admissions with PEFR documented on admission and on discharge. **As recommended by the National Asthma and COPD Audit Programme (NACAP).*

Frequency of admissions vs. admissions with no obvious obstruction

Figure 13 shows the association between the frequency of admissions per patient and having a NOO event. Compared to STA, MTA were more likely to have had an admission with NOO (14.3% vs. 34.2%, P= 0.003) (Table 17). Similarly, there was an increase in the number of patients with a NOO admission in FTA compared to patients with less than 4 admissions (57.1% vs. 16.4%, P= 0.006). In addition, there was a significant trend towards an increased number of patients with NOO admissions with increasing frequency of admissions (P <0.001) (Figure 13).

Figure 13. Frequency of admissions vs. no obvious obstruction admissions.

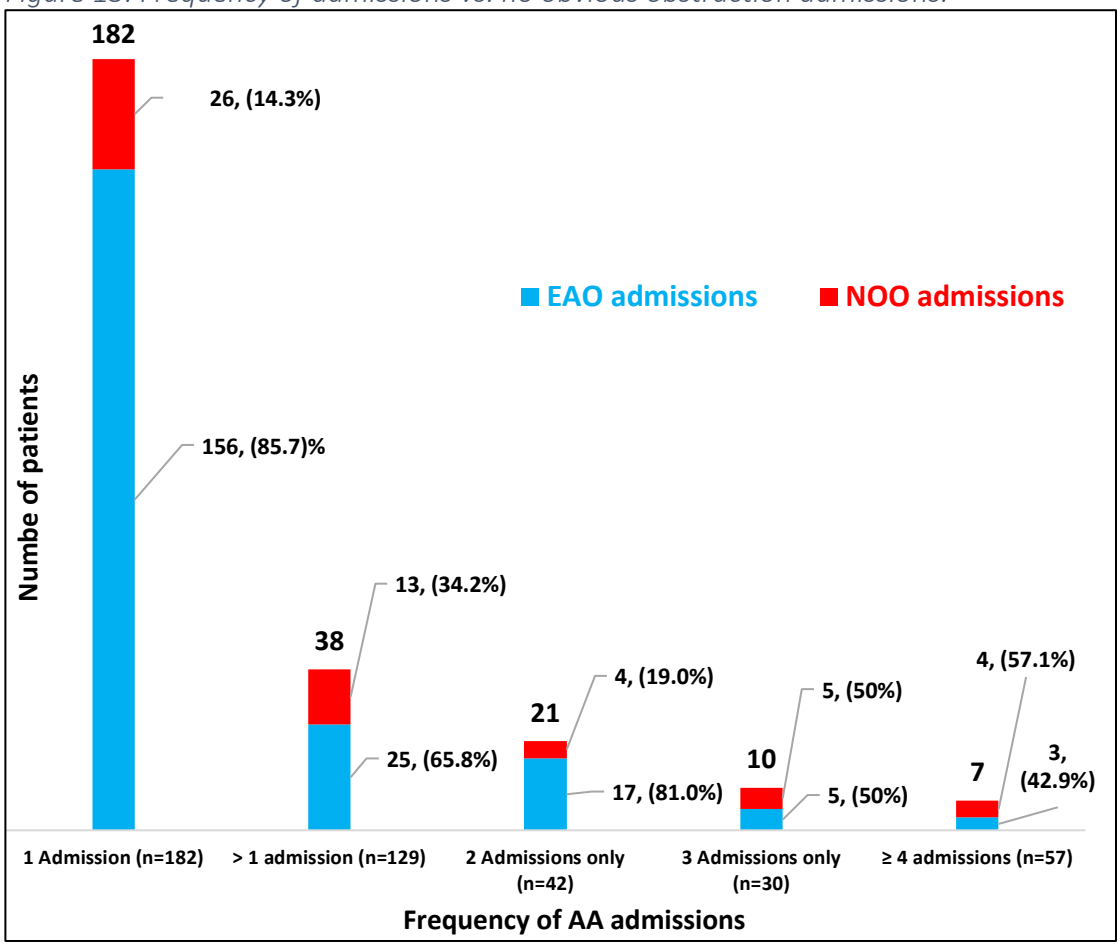


Table 17. No obvious obstruction events categorised according to admission frequency.

	STA	MTA	P value	<4 admissions	FTA	P value
	N (%)			N (%)		
Number of patients	182 (82.7)	38 (17.3)		213 (96.8)	7 (3.2)	
Number of admissions	182 (58.5)	129 (41.5)		254 (81.7)	57 (18.3)	
Patient to visit ratio	1:1	1:3.4		1:3.4	1:8.1	
NOO AA*	26 (14.3)	13 (34.2)	0.003	35 (16.4)	4 (57.1)	0.006

**Ever had no obvious obstruction admission during the audit period.*

Univariate analysis of factors associated with admissions in patients with NOO admissions indicated that asthma mimics (ILO, BPD, and EDAC), age, and the number of admissions were significantly associated with having had an admission with NOO

(Table 18). In the multivariate model, although none of the factors were significantly associated with NOO admissions, age and the number of admissions tended to be the strongest risk factors for having NOO hospital admissions (P= 0.07, P= 0.06, respectively) (Table 18).

Table 18. Logistic model of factors associated with hospital admissions in patients with vs. without no obvious obstruction admissions.

	Unadjusted OR	95% CI	P value	Adjusted OR	95% CI	P value
Gender (females vs males)	1.9	0.8 - 5.2	0.18			
Asthma mimics	3.7	1.3 - 10.5	**0.01**	2.2	0.59 - 6.9	0.21
Previous asthma diagnosis	0.74	0.17 - 5.1	0.71			
Treatment step 4 or 5	0.98	0.46 - 2.01	0.92			
Age (per year increase)	0.97	0.96 - 0.98	**0.03**	0.98	0.96 - 1.0	0.07
Townsend score (per unit increase)	1.1	0.9 - 1.2	0.35			
Number of admissions (per each admission increase)	1.7	1.2 - 2.5	**0.01**	1.5	1.0 - 2.3	0.06

Summary of Appendix B: Audit of asthma attack admissions- Comparison between asthma attack admissions with and without evidence of airflow obstruction only considering first admission of each patient identified

Due to the potential for repeat admissions to influence the independency assumptions of the analyses of AA admissions (the previous section), an additional analysis of admissions, only including the first admission of each patient, was carried out. Results of this additional analysis were largely similar to the results of the main AAs analysis section, and will be briefly summarised below, and the complete analysis will be included in Appendix B.

Of the admissions included in this sub-analysis (N= 220), 34 (15.5%) did not have evidence of airflow obstruction. LOS was lower, and the proportion of those with ITU admissions and secondary diagnoses were higher in the NOO admissions compared to admissions with EAO (2 vs. 3 days, P= 0.07, 5.9% vs. 0.5%, P= 0.06, 64.7% vs. 40.9%, P= 0.01, respectively). Similar to findings from the main admissions analysis, the

proportion of those with increased BEC was lower in the NOO admission (11.8% vs. 25.3%, P= 0.09). Although median BEC was lower in those on pre-admission systemic anti-inflammatory agents (0.01 x10^^{9}/L vs. 0.10 x10^^{9}/L, P= 0.001), the proportion of patients on pre-admission systemic anti-inflammatory treatment was not different between the groups (P= 0.89), and when only including patients not on systemic anti-inflammatory treatment, the proportion of patients with increased BEC was still lower in the NOO admissions (P= 0.09).

Variations in SCS treatment was similar to those observed in the main analysis; NOO admissions tended to be less likely to have received SCS compared to admissions with EAO (94.1% vs. 99.5%, P= 0.06), likely due to the involvement of secondary/contributing diagnoses. Discharge characteristics were similar to those observed in the main admissions analysis.

Discussion

Results from the audit show that a small number of patients repeatedly admitted for acute asthma account for a significant proportion of the overall number of asthma attack admissions. Such patients are well-known to the asthma service, have a higher prevalence of significant comorbidities and asthma mimics, are on high asthma-treatment step, and frequently require asthma-related ITU admissions. Another equally important finding is that a significant proportion of AA admissions had no objective evidence of asthma deterioration, and these are likely due to alternative diagnoses other than AAs; patients with multiple admissions are responsible for a significant proportion of such AA admissions.

Multiple and frequent exacerbators

Our study identified a group of patients with high healthcare utilisation and frequent hospital admissions for AAs. Such patients are likely to be younger females with a lower socioeconomic status, higher asthma treatment step, and higher incidence of asthma mimics compared to single-time attendees. Results from the present study align with findings from previous descriptions of frequent exacerbators, although the definition of "frequent" slightly differs between other studies and ours. Kupczyk et al. provided a detailed description of frequent exacerbators (≥2 exacerbations, defined as deterioration outside the usual daily asthma variation as per the ATS/ERS recommendations; only moderate and severe exacerbations were considered) in moderate and severe asthma patients[436]. Frequent exacerbators in this study were found to be primarily severe asthma patients with lower quality of life and on high-dose ICS compared to non-frequent exacerbators. The latter finding is consistent with findings from the audit and studies demonstrating that asthma patients on high-dose ICS and even oral-corticosteroids treatment are prone to frequent exacerbations[481,482]. Similarly, Ten Brinke investigated factors associated with frequent asthma exacerbations (>3 exacerbations defined as events requiring a course of oral corticosteroids) in a difficult-to-treat cohort; consistent with findings from the present study, frequent exacerbators tended to be younger females with increased prevalence of psychological dysfunction, nasal sinus disease, and obstructive sleep apnoea[390].

Female sex is a previously identified risk factor for asthma exacerbations[155] and frequent AAs[432,483].

The audit also identified that the proportion of those with previously recorded blood eosinophilia (≥0.4 x10^^{9}/L) is significantly higher in those with multiple attendances. Similar findings have been observed in a key study by Price et al., who reviewed medical records of more than 130,000 asthma patients from the UK research database and concluded that blood eosinophilia (≥0.4 x10^^{9}/L), along with anxiety/depression and atopy are associated with ≥2 exacerbations a year[432].

In addition to the common asthma comorbidities noted above, asthma mimics, including ILO and BPD, have also been noted to be increased in multiple and frequent attendees compared to single-time attendees. ILO (commonly referred to as vocal cord dysfunction) is a common asthma mimic that can masquerade as and coexist with asthma[399,404,440,484]. A study describing characteristics of patients with ILO identified a predominantly young female group of patients often misdiagnosed with asthma, with very high asthma-related healthcare utilisation (frequent emergency room visits and hospital admissions), frequent OCS bursts, and on severe asthma treatment[433], similar to the cohort of patients identified in the audit. More recently, a UK-wide registry of patients with endoscopically confirmed ILO similarly described a predominantly female cohort with substantial healthcare utilisation, including emergency visits, hospitalisations, and ICU admissions[404]. Notably, 68% of these patients had coexisting asthma, 14% were undergoing investigations for asthma, and 23% had a diagnosis of BPD.

Similarly, BPD is an asthma mimic[485] that is more prevalent in asthmatics compared to non-asthmatics[486]; BPD has also been associated with young female subjects[412]. BPD and asthma can co-exist[487], and asthma patients with BPD have a lower quality of life compared to those without[488]. In a study aimed at describing the incidence and risk factors of BPD in adults with asthma, lack of asthma control and frequent exacerbations (≥3 events requiring systemic OCS, ED visits, or hospitalisation in the last year) were identified as independent predictors of BPD; the incidence of BPD was significantly higher in patients with severe and brittle asthma[415].

The increased prevalence of comorbidities and mimics in the multiple and frequent attenders group is an increasingly recognised contributor to worsening asthma

outcomes, including exacerbations, in the general asthma population as well as those with severe disease. Tay and colleagues investigated the influence of comorbidities on asthma outcomes in severe asthma patients and concluded that female gender, ILO, and chronic rhinosinusitis were all independent risk factors of ≥3 exacerbations a year, and BPD significantly decreases asthma control[489]. Chronic rhinosinusitis has also been implicated in an increased risk for AAs in patients with less severe asthma[251]; in this study, patients with ≥2 AAs in the previous year were likely to be on higher ICS treatment and to have frequent OCS use and asthma-related admissions and ED visits. It is worth noting that many of these comorbidities can also contribute to ILO[437], which is known to be commonly misattributed to asthma worsening[490], aggravate asthma control[491], and is independently associated with frequent AAs[437].

The study found that the proportion of those requiring intensive care treatment is significantly increased among multiple attendees compared to single-time attendees. The European Network for Understanding Mechanisms of Severe Asthma identified a distinct group of near-fatal asthma patients, previously requiring intensive care for asthma, characterised by sub-optimal treatment compliance[492]; such patients were largely indistinguishable from those with mild-to-moderate asthma in terms of clinical and inflammatory characteristics.

Asthma attack admissions

The audit found that 51 of 311 (16.4%) AA admissions in a year do not have evidence of airflow limitation. Findings from the present study are consistent with a study by Centurion et al. aimed at evaluating the feasibility of spirometry in assessing airflow obstruction in patients hospitalised with asthma and COPD exacerbations. The study found that 17% of the 47 patients admitted for AAs did not have spirometric evidence of airflow obstruction, with flow volume loop abnormalities suggestive of extrathoracic airflow obstruction noted in the admissions with no airflow obstruction; the authors suggested a possible involvement of ILO contributing to the symptoms of such admissions[493]. Although Centurion and colleagues used more robust criteria for assessing evidence of airflow obstruction, the proportion of those without evidence of acute airflow obstruction is similar to the proportion observed in the present study. Further, in a study assessing the rate of false positive AA diagnoses in patients

presenting with acute asthma, Innes[494] reviewed the medical records of 450 patients admitted with acute asthma over a six-month period. PEFR of ≥75% of best or predicted, as well as documentation of other respiratory diseases or asthma mimics, was used to refute the diagnosis of AA. While 78% of those who underwent a respiratory review (n= 347) had no documented evidence to refute the diagnoses of acute asthma, 77 (22%) had their diagnoses refuted by the study team, of whom 19 (25%) had a PEFR of ≥75% and 14 (18%) had documented asthma mimics, including anxiety and hyperventilation. Of note, those with normal PEFR or anxiety and hyperventilation were predominantly younger patients with a female-to-male ratio of 2.4:1, similar to multiple and frequent attenders identified in the present study and in line with previous descriptions of frequent exacerbators[390,432] and those with asthma mimics[404,412,433].

Consistent with findings from studies investigating "frequent asthma exacerbators"[431,495-497], the present study identified a small group of patients responsible for a significant number of cumulative AA admissions. The audit also found that multiple and frequent attendees are responsible for a significant proportion of admissions without EAO. Interestingly, the odds of NOO AA admission per patient progressively increase as the number of admissions increases. This is a novel finding that has not been previously reported. In a study assessing the prevalence of misdiagnosed asthma in frequent exacerbators, Jain and colleagues found that only 38 of the 171 frequently exacerbating patients had previous spirometry testing, and after study enrolment and baseline spirometry testing, 60 (25%) had no evidence of asthma pathology, defined as FEV_1/FVC impairment or positive methacholine challenge test (MCT), of whom 11 (18%) had evidence of ILO pathology[438]. Although normal lung function measures and MCT during periods of clinical stability cannot exclude a diagnosis of asthma, especially in those already on asthma treatment[151], the presence of evidence of extrathoracic abnormality (ILO) in some patients may suggest that their symptoms (during AAs) could have been explained by pathologies other than asthma. It is worth noting that ILO is the most commonly investigated asthma mimic, and some authors have proposed that a significant proportion of asthma patients have laryngeal hyperresponsiveness, which has been associated with ILO[441]. In a study assessing the glottic function in patients presenting to A&E with wheezing and dyspnoea and a

physician diagnosed AA, 10.4% of the 48 patients had laryngoscopic evidence of ILO. Of note, 9 patients had normal spirometry (FEV_1/FVC ≥0.75), of whom 1 had confirmed vocal cord abnormalities, and 8 had laryngoscopic findings suggestive of ILO[401].

In another study assessing the utility of FeNO in discriminating between frequent exacerbators with and without airflow obstruction (defined as FEV_1/FVC impairment or positive MCT), 86 of 222 (38.7%) patients had no evidence of airflow obstruction with a significantly increased number of females in the no airflow obstruction group compared to those with airflow obstruction (77% vs. 59%, P= 0.02); of those with no airflow obstruction, 40% had comorbid diseases including depression, anxiety, and ILO[498].

Although it is plausible that severe asthma patients and those with multiple comorbidities utilise healthcare more frequently, whether such healthcare utilisation is actually asthma-related is a key question. Studies have consistently shown that asthma is commonly over-diagnosed[499], and that many people treated for asthma do not actually have asthma[438,500]. In addition, respiratory symptoms commonly linked to asthma can be easily related to many other pulmonary[476] and extra-pulmonary[403,433] conditions. Objective evidence of airflow obstruction assessment should always be utilised for patients presenting with symptoms suggestive of airflow impairment. Studies have consistently demonstrated the feasibility of spirometry for the assessment of acute airflow obstruction[493,501,502]. However, spirometry is not commonly utilised during acute presentation of respiratory symptoms despite evidence suggesting its utility in differentiating between deterioration of asthma and common asthma mimics[503].

Management of AA admissions

In agreement with previous studies[497,504], the audit noted sub-optimal compliance with AA management recommendations and guidelines, and a significant proportion of patients did not receive optimal asthma care. In a retrospective audit assessing the management of AAs at A&E, only 50% of the admissions had patients' best PEFR documented, and 48% had pre-discharge PEFR recorded. Similar findings have been reported for hospitalised AA patients. In 1995, the British Thoracic Society's (BTS) Standards of Care Committee conducted a national audit assessing the management of

hospitalised AA patients. The BTS audit identified deficiencies in each aspect of care, including PEFR measurement on admission and prior to discharge, outpatient follow-up arrangement, and follow-up visit attendance[478]. Similar findings have also been reported in more recent studies[505,506].

An interesting finding is that the number of patients who received the six elements of good practice care tended to be decreased in the patients with NOO, an unanticipated finding given that many of these admissions were of frequent attenders. Whether this is because these patients have been previously admitted and seen is difficult to determine, but this finding is in keeping with findings from studies of frequent exacerbators[497].

Study strengths and limitations

This study had multiple strengths. First, although the audited AA admissions were based on ICD-10 coding diagnoses, which has been previously reported to be inaccurate[507], actual diagnoses were confirmed from medical notes and discharge summaries, and admissions with discordance of ICD-10 codes and actual documented diagnosis were excluded (Figure 9), minimising any imprecision that could arise from inaccurate ICD-10 coding. Second, only admissions to the respiratory admission units were included, limiting the audited admissions to those who received an AA diagnosis following a specialist respiratory review. Another important strength of the present study is that the number of AA admissions audited was high; although this is not a powered study, findings from this number of cases should lead to meaningful conclusions from the population studied.

Limitations to this study do exist. The retrospective nature of this study and the reliance on data manually entered into medical records could confound the accuracy of collected data[508]. Data collected for this audit is largely dependent on the attending physician's documentation and accuracy and completeness of documented data; it is likely that missing data, such as PEFR level on admission, was performed but not documented. As such, the potential for information bias must be considered. Moreover, missing data were present for several key variables, including PEFR. Although this is unlikely to bias the overall direction of findings, its impact on subgroup analyses and variable comparisons cannot be entirely excluded. Also, again, due to its

retrospective nature, the study relied on the least objective measures of airflow obstruction (wheeze and PEFR) compared to more objective measures (spirometry and forced oscillation). However, this is an attempt to describe and phenotype AA admissions based on available electronic health records data, and the measures used to define airflow obstruction in this study are the standard of care commonly used in clinical practice. Given the inherent difficulty in distinguishing between loss of asthma control, AAs, and conditions that mimic AAs, it is entirely possible that the measures used to define airflow obstruction (respiratory physician assessment and PEFR measurement) miscategorised a number of events due to the 'diagnostic overshadowing' effect[509] of a previous asthma diagnosis[433] and the potential tendency of clinicians to diagnose and treat events as asthma attacks to avoid potentially serious consequences of undertreated acute asthma, even if this is clinically unlikely. Another limitation is that not all AA admissions were audited. However, the study team believes that the sample size (220 patients, 311 admissions) was representative, and the exclusion of non-audited admissions is unlikely to significantly influence the study findings. Further, as all the audited admissions were of patients admitted to the respiratory admission unit, there is a potential that some admission to other units were missed. However, as illustrated in Figure 9, only a small number of admissions were admitted to A&E and medical units other than respiratory. Hence the potential for selection bias exists, but given the small number of cases excluded this is unlikely to have significantly influenced the study findings. While this approach ensured specialist review and diagnostic consistency, it may under-represent milder cases or more complex patients potentially admitted elsewhere. Likewise, due to the retrospective study design, it is likely that the study team have missed admissions incorrectly not coded as asthma attacks. In addition, the number of patients in the frequent-time attendees group was small. While this group was analysed categorically, alternative approaches such as modelling as a continuous variable or using tertiles were considered. However, low numbers limited the statistical power for such subgroup analyses. This comparison (single-time attendees vs. frequent-time attendees) is presented as exploratory analysis rather than as core analysis. Moreover, although a well-established blood eosinophil count cutoff (≥0.4 $x10^9$/L) is utilised in the present study, there is a potential for missing subjects with a cutoff at the upper 95% CI;

however, this is a common limitation of asthma attack studies as attack-specific cutoffs are not well established. In addition, although the primary analysis was descriptive, the chapter included multiple statistical comparisons, raising the potential for type I error (false positives). No adjustment was made for multiple testing, given the exploratory nature of the study; however, all p-values should be interpreted with caution, particularly where findings approached significance. Further, this is a single-centre study, and the majority of patients identified were of a White/British ethnicity; therefore, findings might not be generalisable to other populations. Finally, this is a descriptive retrospective analysis of AAs using electronic health records data; thus, a causative relationship cannot be established.

Conclusion

Although the characteristics of frequent attenders are well understood, details of their AA admissions have not been previously investigated. Given the overlap of the characteristics between frequent exacerbators and those with asthma mimics, it is plausible to postulate that presumed AAs in certain groups of patients, including those with severe disease, comorbidities, and frequent admissions, may be due to non-asthma pathology. This retrospective clinical audit of medical records of 311 AA admissions found that a significant proportion of AA admissions do not have documented evidence of airflow limitation. The study also identified a sub-group of patients repeatedly admitted for AAs, who are responsible for a significant number of AA admissions in a year. These patients were likely to have comorbidities, asthma-like conditions, and AA admissions lacking objective evidence of airflow limitation.

Confirming the findings of this audit is important, and identifying such patients is of paramount significance. The utility of spirometry and other point-of-care measures for assessing airflow obstruction in potential AAs requires assessment. In addition, the utility of AA inflammatory profiling in identifying such patients is attractive, as one of the earliest descriptions of patients with ILO identified a significant lack of eosinophilia in the ILO group compared to the asthma group[433]. Findings from this study informed the design of a subsequent prospective study described in the following chapters.

Chapter 3: APEX3- Cohort overview and clinical characteristics

Introduction

Asthma attacks are significant events associated with substantial healthcare expenditure[510], significant impact on quality of life[511], permanent loss of lung function[84], hospitalisation and even death[386,510]. There is a lack of consensus over the definition of AAs, and as a result, these events are poorly defined, and the distinction between AAs and poorly controlled asthma is difficult. Furthermore, the pathophysiology and heterogeneity of asthma attacks are poorly understood, and the measurement of inflammatory biomarkers proven to be useful in the assessment of stable asthma has not been evaluated in the setting of acute asthma. Systemic corticosteroids, first used for AAs in the 1950s[167], remain the cornerstone treatment option despite concerns regarding their overuse, potentially variable efficacy[512-514], and well-documented toxicity[206,207,209,211]. In addition, asthma is commonly overdiagnosed[500], the specificity of respiratory symptoms for asthma is limited[476], and confirmation of airflow obstruction during AAs is often overlooked[515-518]. In fact, the most commonly used measure of airflow obstruction, peak expiratory flow rate, may not always be reliable[519-521], largely due to its requirement for maximum patient effort and cooperation[522-524], and its well-recognised dissociation with asthma symptoms[525]. As a result, many diseases presenting with asthma-like symptoms can be easily mistaken for acute asthma[526]. Additionally, previous studies on asthma attacks have primarily focused on large airway involvement, with little attention paid to small airway dysfunction or the underlying inflammatory mechanisms. While the contribution of these components during acute episodes remains unclear, there is a potential that consideration of such mechanisms might help distinguish true asthma attacks from episodes of out-of-control asthma (raised inflammatory markers and intact lung function) and those due non-asthma related pathologies (normal inflammatory markers and intact lung function). However, this area has yet to be systematically explored.

Whilst the importance of large airway pathology has long been recognised in asthma, there is a growing appreciation that small airway dysfunction is also an integral part of the asthmatic disease process[114]. Studies have consistently demonstrated that SAD is

highly prevalent in asthma patients, with estimates ranging between 50% and 90%[97-100]. The extent of small airways involvement in the pathophysiological mechanisms of AAs is not well understood, but evidence implicating the small airways in the symptomatology of asthma is convincing. The proportion of AAs secondary to dysfunction of the small airways is not clear and current asthma guidelines do not include measures for the assessment of SAD.

APEX3 is a real-world prospective, observational study describing asthma attacks on admission to the hospital and during recovery. This chapter will provide an overall description of patients recruited and admissions identified and the characteristics of their attacks including measures of lung function and inflammation.

Aim

To describe the clinical and demographic characteristics of patients admitted to hospital with suspected severe acute asthma.

Primary objective

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

Secondary objectives

- Determine the proportion of patients hospitalised with suspected asthma attacks likely due to non-asthma pathology.
- Describe and assess the incidence of extra-pulmonary co-morbidities in patients hospitalised with asthma attacks.
- Assess incidence of risk factors for attacks based on patients' previous history.
- Assess patients' adherence with asthma medications.
- Compare the use of spirometry and forced oscillation technique (FOT) as objective measures of airflow obstruction in suspected severe asthma attacks to the standard measure of airflow obstruction peak expiratory flow rate.
- Compare the use of fractional exhaled nitric oxide as a measure of airway inflammation against sputum and blood eosinophils.
- Describe objective and symptomatic treatment response and recovery from severe asthma attacks following hospital discharge.

Hypotheses

1. A proportion of patients admitted to hospital with suspected asthma attacks have an alternate cause for their clinical presentation.
2. The 'phenotypes' of asthma attacks demonstrate significant variation in terms of measures of lung function, inflammatory profile, infection status, and response to treatment.

Methods

Study design

A single-centre, longitudinal, observational study.

Ethical Approval

The study was reviewed and approved by the Research Ethics Committees Northern Ireland B (Ref. 22/NI/0003) and Nottingham University Hospitals (NUH) NHS Trust Research and Innovation (Ref. 22RM002).

Study Population

Eligibility criteria

Inclusion Criteria

- Male or female aged ≥ 18 years.
- Admission with a suspected acute asthma attack.

Exclusion criteria

- Other clinically significant respiratory diseases.
- Any other clinically significant medical disease or uncontrolled concomitant disease.
- Pregnant women, lactating women or women who are planning to become pregnant.
- Investigator determined apparent other cause for admission.
- Acute COVID infection.

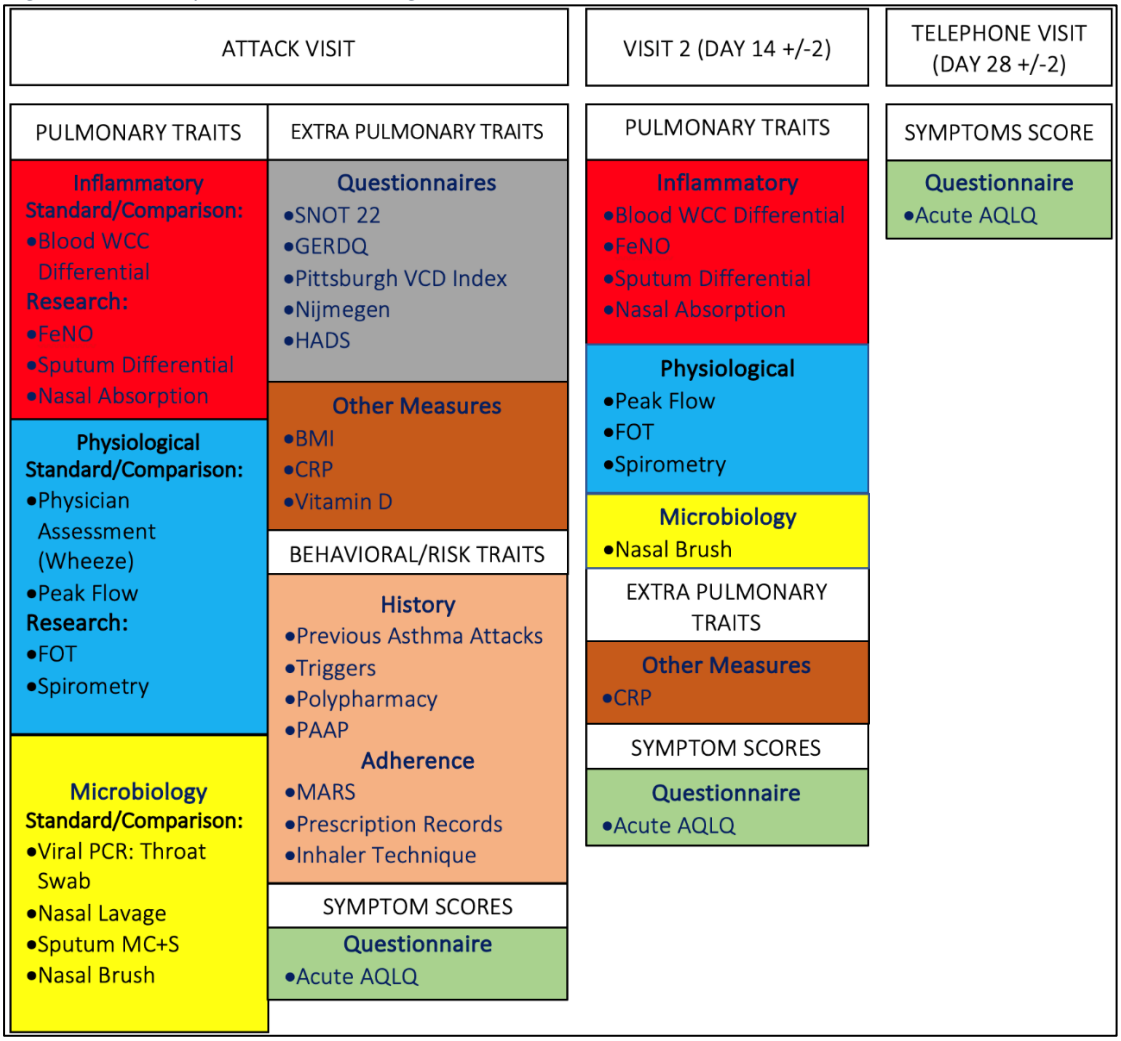
Study setting and participant recruitment

The study was conducted at Nottingham University Hospitals NHS Trust. Patients admitted to the respiratory admission unit, either directly or through A&E, with a primary admission diagnosis of asthma exacerbation were prospectively identified and recruited. A range of investigations, including lung function tests, blood tests, and questionnaires were performed (Figure 14).

A follow-up visit was arranged, which took place approximately two weeks after hospital discharge at the Nottingham Respiratory Research Unit, at which further lung function tests, blood tests and questionaries were performed (Figure 14).

A subsequent telephone follow-up visit was scheduled around four weeks after hospital discharge, at which additional questionnaires were performed to determine the degree of symptomatic recovery (Figure 14).

Figure 14. Study assessments algorithm.



Outcome measures

As this is an observational study with a primary objective that is descriptive in nature, all outcome measures are equally essential in describing both primary and secondary objectives; outcome measures include:

- A specific asthma history (polypharmacy, previous asthma attacks, triggers, personalised asthma action plan) for the assessment of risk factors for attacks.
- Assessment of airflow obstruction at attack and recovery visits using spirometry, Forced oscillation technique, peak expiratory flow rate, and physician recorded breath sounds.
- Assessment of airway inflammation at attack and recovery visits using fractional exhaled nitric oxide, sputum inflammatory cell count, and blood eosinophil count.
- Assessment of cytokine levels in nasal lining fluid at attack and recovery visits.
- Assessment of evidence of infection: Throat swab/nasal lavage for viral PCR, sputum for bacterial culture and sensitivity, and C-reactive protein.
- Assessment of symptom burden at attack and recovery visits using the Acute Asthma Quality of Life Questionnaire (AAQLQ).
- Assessment of incidence of extra-pulmonary co-morbidities: Questionnaires (Sino-nasal Outcome Test (SNOT-22)[527], Gastro-esophageal reflux disease questionnaire (GerdQ)[528], Pittsburgh vocal cord dysfunction index[484], Nijmegen questionnaire for dysfunctional breathing[529], Hospital anxiety and depression scale)[530], body mass index (BMI), and vitamin D.
- Assessment of adherence to medications (Medication adherence report scale[531], prescription records, inhaler technique) for the assessment of behavioural attack traits.

Sample size calculation

This is an observational, hypothesis-generating study and is a part of PhD project; as such, an achievable sample size of 100 patient was set.

Summary of study protocol

A copy of the protocol is included in Appendix C.

Visit 1 (V1)

The initial assessment took place at the earliest timepoint possible following hospital admission. A range of investigations were performed including lung function tests, blood tests, and questionnaires (Figure 14).

Visit 2 (V2)

A follow up visit was arranged two weeks following hospital discharge during which most of the study assessments conducted at V1 were repeated (Figure 14).

Visit 3 (V3, telephone follow-up)

A telephone follow up was arranged four weeks following hospital discharge during which the Acute Asthma Quality of Life Questionnaire was performed (Figure 14).

Data collection

Medical history and concomitant medications

Medical history and asthma medications were collected from each patient. Patient reported pre-admission OCS use was recorded. Pre-admission OCS use was also confirmed for all patients using prescription records.

Clinical Measurements

Lung function measures

Spirometry

Spirometry was performed using a handheld spirometer (Easy-on, ndd medical, Zurich, Switzerland). Device calibration, as well as reliability and repeatability assessments, were carried out in accordance with the American Thoracic Society/European Respiratory Society (ATS/ERS) recommendations[532]. Predicted normal values were calculated using the Global Lung Function Initiative (GLI) 2012 equations[533].

Forced oscillation technique

Forced oscillation technique was performed using the TremoFlo oscillometry device (Thorasys, Canada). Device calibration, as well as reliability and repeatability assessments, were carried out in line with the 2019 ERS taskforce recommendations

for respiratory oscillometry[534]. Due to the potential impact of spirometry on bronchomotor tone[535], FOT was always performed prior to spirometry.

Assessment of breath sounds

Breath sounds were assessed during all admissions by the attending respiratory consultant and were recorded on the study case report form.

Inflammatory measures

Blood eosinophil count

Blood samples for blood eosinophil count were collected into 4 mL EDTA tubes using 21-gauge Vacutainer® Safety-Lok™ blood collection kit (BD, Plymouth, UK). Blood samples were processed at NUH laboratories following local protocols.

Fraction of Exhaled Nitric Oxide

Fractional exhaled nitric oxide was measured using a handheld device (Niox Vero, Aerocrine, Sweden) at an exhaled flow rate of 50 mL/s for a minimum of 10 seconds. Device calibration, as well as reliability and repeatability assessments, were carried out according to the American Thoracic Society/European Respiratory Society recommendations[536]. FeNO measurement was performed prior to forced oscillation, spirometry, and sputum induction as these may influence FeNO values.

Sputum eosinophils and sputum induction

Due to the potential of sputum induction to induce bronchospasm, the investigator always attempted to obtain a spontaneously expectorated sputum sample. If a patient was not able to spontaneously produce sputum, sputum induction was then carried out using a previously published protocol[537] (Appendix D). Briefly, patients were pre-treated with 2.5 mg nebulized salbutamol followed by FEV_1 measurement. If post bronchodilator FEV_1 was ≥1 L or ≥40% of predicted, patients were transferred to the Respiratory Research Unit for sputum induction if permitted by the attending respiratory consultant. Sputum induction was not performed if any of the following occurred at any point during the admission:

- Any features of life-threatening attack as per the BTS[184] guideline: (PEFR <33% best or predicted, SpO2 <92%, PaO2 <8 kPa, 'normal' PaCO2 (4.6–6.0 kPa), altered conscious level, exhaustion, arrhythmia, hypotension, cyanosis, silent chest, poor respiratory effort).
- Requiring supplemental oxygen to maintain SpO2 ≥94%.
- Patient, clinical team or investigator opinion that patient unable to tolerate procedure.

Following transfer to the Respiratory Research Unit, 0.9% saline was administered using an ultrasonic nebuliser for 30 seconds, 1 minute, and two 2-minute periods with FEV_1 measured 1 minute after each nebulisation period. Sputum induction was ceased if a sputum sample was obtained or if FEV_1 fell ≥20% of pre-induction baseline. Sputum obtained was stored in ice and processed within 2 hours as previously described by Pavord *et al.*[538]. Sputum samples were processed for sputum differential cell count at the University of Nottingham (UoN) laboratories following local protocols.

Cytokines profiling and nasal absorption

A fibrous hydroxylated polyester absorptive matrix filter paper (Leukosorb) was used for the collection of nasal lining exudates as previously described[539]. Two, 7mm by 30mm strips placed one on top of the other were placed in the anterior portion of the inferior turbinate of one nostril using blunt ended forceps and secured in place using nasal clips for a total of two minutes. Leukosorb strips were then removed and placed into a cryovial containing 300µl elution buffer and placed on ice until processing. Leukosorb strips were then washed in a buffer containing Triton-X 1% and BSA 1% and then centrifuged for 20 minutes at 16,000g at 4°C. Aliquots were then frozen in a cryogenic tube at −80 °C.

A multiplex analysis was performed for interleukin (IL) 5, IL-13, IL-4, IL-33, Thymic stromal lymphopoietin (TSLP), IL-17, IL-8, IL-1β, IL-6, Tumour Necrosis Factor (TNF) α, Interferon (IFN) α, IFN-γ, and IFN-β using a Luminex (Bioplex 200 array reader) analyser (Luminex corporation, Austin, Texas, USA) according to manufacturer's instructions.

Infection measures

Sputum microscopy, culture and sensitivity

Sputum samples obtained, either induced or spontaneous, were processed for bacterial culture and sensitivity at Nottingham University Hospitals laboratories following local protocols.

Throat swabs for viral identification

Respiratory virus PCR throat swabs were obtained and analysed by NUH staff members following local protocols. Results were recorded on the study case report form.

Full blood count and c-reactive protein

Blood samples for full blood count and c-reactive protein were collected into 4 mL EDTA and 5mL serum tubes, respectively, using 21-gauge Vacutainer® Safety-Lok™ blood collection kit (BD, Plymouth, UK). Blood samples were processed at NUH laboratories following local protocols.

Symptoms score

The Acute asthma quality of life questionnaire is a modified version of the Asthma quality of life questionnaire designed to assess health-related quality of life during acute exacerbation of asthma[540,541], and is frequently used in acute asthma studies to assess acute asthma-related quality of life[542,543]. The AAQLQ is an 11-item, interviewer-administered questionnaire consisting of two domains: symptoms and emotional, and is scored on a 7 scale Likert scale at each question with a lower AAQLQ score indicating a higher level of distress. The minimal important difference of the AAQLQ is 0.5[544].

Extra-pulmonary comorbidity questionnaires

Sino-nasal Outcome Test-22

The incidence of upper airway symptoms was assessed using the Sino-nasal Outcome Test-22 (SNOT-22) questionnaire. SNOT-22 is a validated, 22-item questionnaire for the assessment of upper airway and nasal symptoms and health-related quality of life impact of chronic sinusitis[527]. SNOT-22 comprises 5 categories: nasal (8 items), emotion (3 items), function (3 items), sleep (4 items), and facial/ear (4 items), with items scored

on a scale of 0 (no problem) to 5 (problem as bad as it can be) and the overall SNOT-22 score being the mean of the items in each domain ranging from 0 to 110; scores of the SNOT-22 questionnaire are stratified into mild (8-20), moderate (>20-50), and severe (>50).

Gastro-esophageal reflux disease questionnaire

The gastro-esophageal reflux disease questionnaire (GERDQ) is a widely used, patient reported questionnaire for the assessment of reflux symptoms severity[528]. GERDQ consists of 6 questions evaluating 4 positive predictors and two negative predictors. Questions are evaluated on a scale of 0 to 3 with 0 being little complaints and 3 being severe complaints. The overall score of the GERDQ range from 0 to 18 with total score of 0 to 2 indicating 0% likelihood, 3-7 indicating 50% likelihood, 8-10 indicating 79% likelihood, and scores from 11-18 indicate 89% likelihood of the disease.

Pittsburgh Vocal Cord Dysfunction Index

The Pittsburgh vocal cord dysfunction index (PVCDI) is a simple four item questionnaire (wheezing: 2 points, throat tightness: 4 points, dysphonia: 2 points, and whether symptoms are triggered by odours: 3 points) designed to distinguish asthmatics from those with vocal cord dysfunction (now called inducible laryngeal obstruction)[484]. A weighted score of ≥4 indicates an increased likelihood of VCD with a sensitivity of 83% and a specificity of 95%.

Nijmegen Questionnaire

The Nijmegen questionnaire is a validated, 16-item, self-administered tool commonly used for the diagnosis of Hyperventilation syndrome (HVS)[529]. Each of the questions assesses the frequency of HVS on a scale of 0-5 with 0 being never and 4 being very often. A cutoff score of >23 is considered significant and indicates an increased likelihood of HVS.

Hospital Anxiety and Depression Scale

The Hospital Anxiety and Depression Scale (HADS) is a patient reported screening tool commonly used to assess the presence of anxiety or depression[530]. The HADS questionnaire consists of 14 questions divided into two 7-items sub-scales, one for

anxiety and one for depression, with each question rated on a 4-point Likert scale with a maximum score of 21 for each domain. Scores between 0-7 are considered normal, 8-10 are considered borderline, and scores ≥11 indicate increased likelihood.

Body mass index

Body mass index was obtained from patients' admission records.

Vitamin D

Blood samples for the assessment of vitamin D level were collected into 5 mL serum tubes using 21-gauge Vacutainer® Safety-Lok™ blood collection kit (BD, Plymouth, UK). Blood samples were processed at NUH laboratories following local protocols.

Risk and behavioural traits

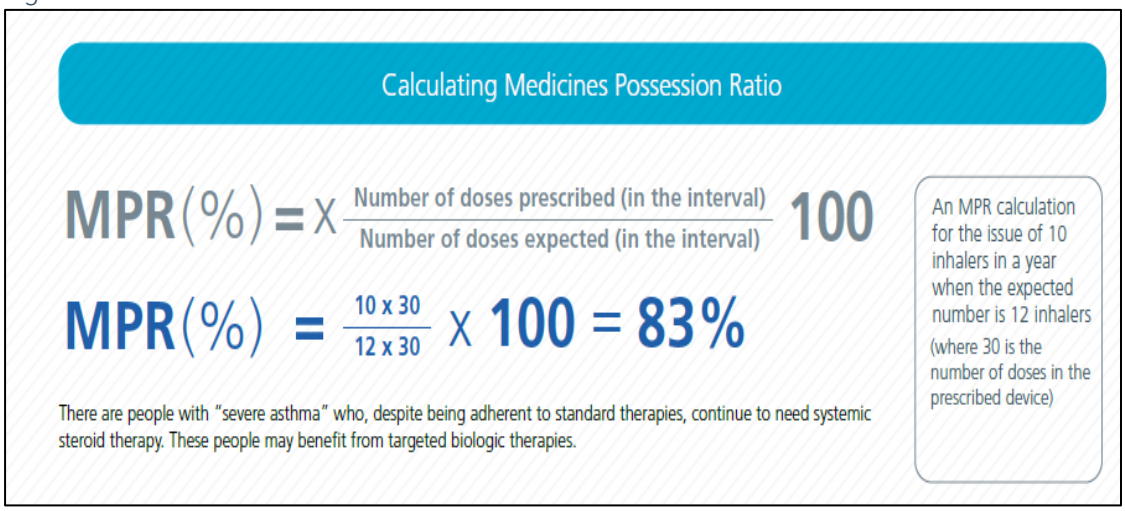
Medication Adherence Report Scale-5

The medication adherence report scale-5 (MARS-5) is a validated, shortened version of the medication adherence report scale-10[531]. It comprises 5 questions assessing non-adherence behaviours, each assessed on a 1 (always)-5 (never) Likert scale with an overall score ranging from 5 (lowest adherence) to 25 (maximum adherence).

Medicines Possession Ratio

Adherence to ICS was assessed using the Medicines Possession Ratio (MPR%) following local Procedure for carrying out adherence checks for the severe asthma service. The MPR% is a calculation comparing the number of inhalers a patient is expected to be issued to the actual number of inhalers issued in a given period of time (Figure 15). Data on medication prescription were accessed using relevant electronic prescription records (SystmOne or CareCentric). A cutoff of 75% was used to distinguish optimal and sub-optimal medication adherence as per local protocols, and as used in previous studies[545-547].

Figure 15. Medicines Possession Ratio calculation.



Inhaler technique assessment

Non-intentional non-compliance with inhalers was assessed using the National Health Service (NHS) Liverpool Clinical Commissioning Group inhaler technique checklist as previously used in asthma studies[548].

APEX3 criteria

The following criteria were used to define and categorise patients into distinct subgroups based on the presence of airflow limitation suggestive of acute asthma and elevated inflammatory markers indicative of active disease. While these criteria were adapted for the purpose of this study and are not formally established in existing guidelines, they are based on sound clinical reasoning and supported by the best available evidence to date.

- *Likely Acute Asthma-Related Admissions* (LAARA) based on the presence of airflow limitation, a prominent feature of acute asthma.
- *Likely Not Acute-Asthma Admissions* (LINAA) based on the absence of airflow limitation.
- *Likely Out of Control Asthma Admissions* (LOCA) based on the absence of evidence of airflow limitation but raised inflammatory markers indicative of active disease state.
- *Non-Asthma-Related Admissions* (NARA) based on the absence of airflow limitation and inflammatory markers within normal levels.

Evidence of acute asthma

Assessment of evidence suggestive of acute asthma was based on the presence of objective evidence of airflow limitation using at least one of the following criteria:

1. Forced expiratory volume in 1 second (FEV_1)/Forced vital capacity (FVC) ratio of less than 0.7 as per the Global Initiative for Asthma 2020[151], as used in previous studies[493,498].
2. Resistance at 5 Hz (R5) of greater than 5 $cmH_2O/L/s$ as per Lipworth's criteria for defining clinically relevant degree of airflow obstruction[549].

Classification of inflammatory markers

For the assessment of inflammatory profiles, the following criteria were used.

T2-high inflammation

The following criteria will be referred to as $T2^{APEX3}$ and will be used as the main criteria to define T2-high inflammation in the study:

- FeNO level ≥25 ppb, as used in previous asthma clinical trials[550], and in-line with the American Thoracic Society[551] (ATS) and the National Asthma Education and Prevention Program Coordinating Committee[552] (NAEPPCC) recommendations for elevated T2-high inflammation and/or
- Blood eosinophil count of ≥0.4 $x10^9$/L. This cutoff was used as it is above the threshold of normal blood eosinophil range (0 – 0.4) for clinical practice in the UK[553] and is commonly used to define eosinophilia in asthma studies[467,554] and/or
- Sputum eosinophil count of ≥3%, a well-established cutoff commonly used to define eosinophilic airway inflammation in asthma studies[239,429,555].

GINA criteria for persistent T2-high inflammation[556] ($T2^{GINA}$) were used as exploratory cutoffs as previously used in clinical trials[455,457,557]:

- Blood eosinophils ≥0.15 $x10^9$/L, and/or
- FeNO level of ≥20 ppb, and/or
- Sputum eosinophils ≥2%.

Neutrophilic inflammation

Sputum neutrophil count (SNC) of ≥65%[39] as used in previous studies to define neutrophilic airway inflammation[455,557,558].

Overall inflammatory profiles were classified into 4 categories as follows:

- Eosinophilic: elevated $T2^{APEX3}$ (BEC ≥0.4 $x10^9$/L, or FeNO ≥25 ppb, or SEC ≥3%) and <65% sputum neutrophils.
- Neutrophilic: normal $T2^{APEX3}$ (BEC <0.4 $x10^9$/L, and FeNO <25 ppb, and SEC <3%) and ≥65% sputum neutrophils.
- Mixed phenotype: elevated $T2^{APEX3}$ (BEC ≥0.4 $x10^9$/L, or FeNO ≥25 ppb, or SEC ≥3%) and ≥65% sputum neutrophils.
- Paucigranulocytic: normal $T2^{APEX3}$ (BEC <0.4 $x10^9$/L, and FeNO <25 ppb, and SEC <3%) and <65% sputum neutrophils.

Data management and analysis

Data were prospectively collected and stored in a secured web-based server (REDCap, Research Electronic Data Capture)[480]. Microsoft Excel was used for data management and cleaning. Data analysis was carried out using R version 2024.04.2+764 (R Foundation for Statistical Computing, Vienna, Austria)[559], SPSS version 29.0.2.0 (IBM Corp, 2024), and GraphPad Prism (GraphPad Software, California, USA).

Missing data

Missing data were present across several physiological and inflammatory variables, including spirometry parameters (FEV_1, FEV_1/FVC), forced oscillation technique (R5, R5–R20), and inflammatory biomarkers such as FeNO, blood eosinophil count (BEC), and sputum eosinophil count (SEC). Missingness mainly arose due to a combination of factors: patient inability or contraindication to perform lung function testing, technical difficulties, sample quality issues, and in some cases, logistical or operational constraints (availability of scientists to process samples). No imputation was applied for missing values. All analyses were conducted on a completed-data basis, with each variable or comparison based on the number of available observations. Denominators are reported throughout tables to reflect the actual number of observations contributing to each analysis or subgroup comparison.

Statistical analysis

Continues variables were expressed as mean (SD) for normally distributed data and median (IQR) for skewed distribution. Distribution of data was visually assessed. Categorical data were expressed as numbers and percentages. Number of observations presented will vary depending on available data.

Association between time spent in hospital before study assessment and lung function

To assess whether time spent in the hospital prior to study recruitment had an influence on lung function Spearman's correlation coefficient was performed between lung function measures (FEV_1%, FVC%, FEV_1/FVC, PEFR%, and R5) and the time spent prior to study recruitment.

APEX3 vs. the standard of care measure of airflow obstruction

To assess the utility of peak expiratory flow rate, as the commonly used measure to assess airflow limitation in asthma patients, in the identification of airflow obstruction as defined per the study methodology, a receiver operating characteristic (ROC) curve analysis was carried out to determine optimal PEFR cutoff and to calculate Youden index score, sensitivity, specificity, negative predictive value (NPV), and positive predictive value (PPV).

Predicting airflow obstruction as defined per the study methodology

To determine factors predicting airflow obstruction as defined using APEX3 methodology, a stepwise multivariate logistic model was carried out using R. Factors included are BEC ≥0.4 $x10^9$/L, FeNO ≥25 ppb, FeNO >50 ppb (note: only one FeNO cutoff was included in the multivariate model; if both were significantly associated with the outcome in univariate analysis, the more strongly associated cutoff was selected), PEFR <75%, wheezing, age, gender, AAQLQ score, OCS for asthma in the last 12 months. Factors significantly associated (<0.05) with the outcome in the univariate logistic model were included in the multivariate model.

Association between T2 parameters

To assess the utility of point-of-care (POC) T2-high biomarkers in predicting the "gold standard" T2-high biomarker, sputum eosinophilia (SEC ≥3%), the correlation between BEC and SEC was assessed using Spearman's correlation coefficient and a ROC curve analysis was carried out to identify the optimal BEC cutoff to identify a SEC ≥3%. Similarly, the correlation between FeNO and SEC was assessed using Spearman's correlation coefficient, and a ROC analysis was performed to assess the optimal FeNO cutoff in identifying SEC ≥3%.

Predicting sputum eosinophils ≥3%

To assess the utility of POC markers in predicting sputum eosinophilia, a stepwise multivariate logistic model was carried out using R. Factors included are BEC ≥0.4 $x10^9$/L, FeNO ≥25 ppb, FeNO >50 ppb (note: only one FeNO cutoff was included in the multivariate model; if both were significantly associated with the outcome in univariate analysis, the more strongly associated cutoff was selected), PEFR <75%, FOT R5 ≥5 $cmH_2O/L/s$, FEV_1/FVC <0.7, wheezing, age, gender, AAQLQ score, OCS for asthma in the last 12 months. Factors significantly associated (<0.05) with the outcome in the univariate logistic model were included in the multivariate model.

Results

Admissions identified and eligibility criteria

Between the period from 13th May 2022 and 20th October 2023, 158 patients were identified and approached, of whom 30 declined to participate, 28 did not meet the eligibility criteria, and 100 agreed and consented to participate in the study (Figure 16), of whom 39 (39%) were directly admitted to the respiratory admissions unit and 61 (61%) were admitted through A&E. Of those who consented, 1 patient withdrew soon after consenting (early withdrawal; no data was collected), and 2 patients participated twice in the study on different occasions, which was acceptable as per protocol. For those who participated twice, only the first study participation was considered in this analysis. Overall, therefore, 97 patients are included in the study analysis.

In-hospital asthma attack admission visit (visit 1)

Overall, 99 patients completed visit 1 (V1). As described above, 2 patients were recruited for the study on two different admissions; the second study participation for those patients was removed from this analysis. Thus, although 99 V1s were completed, only 97 were included in the main analysis.

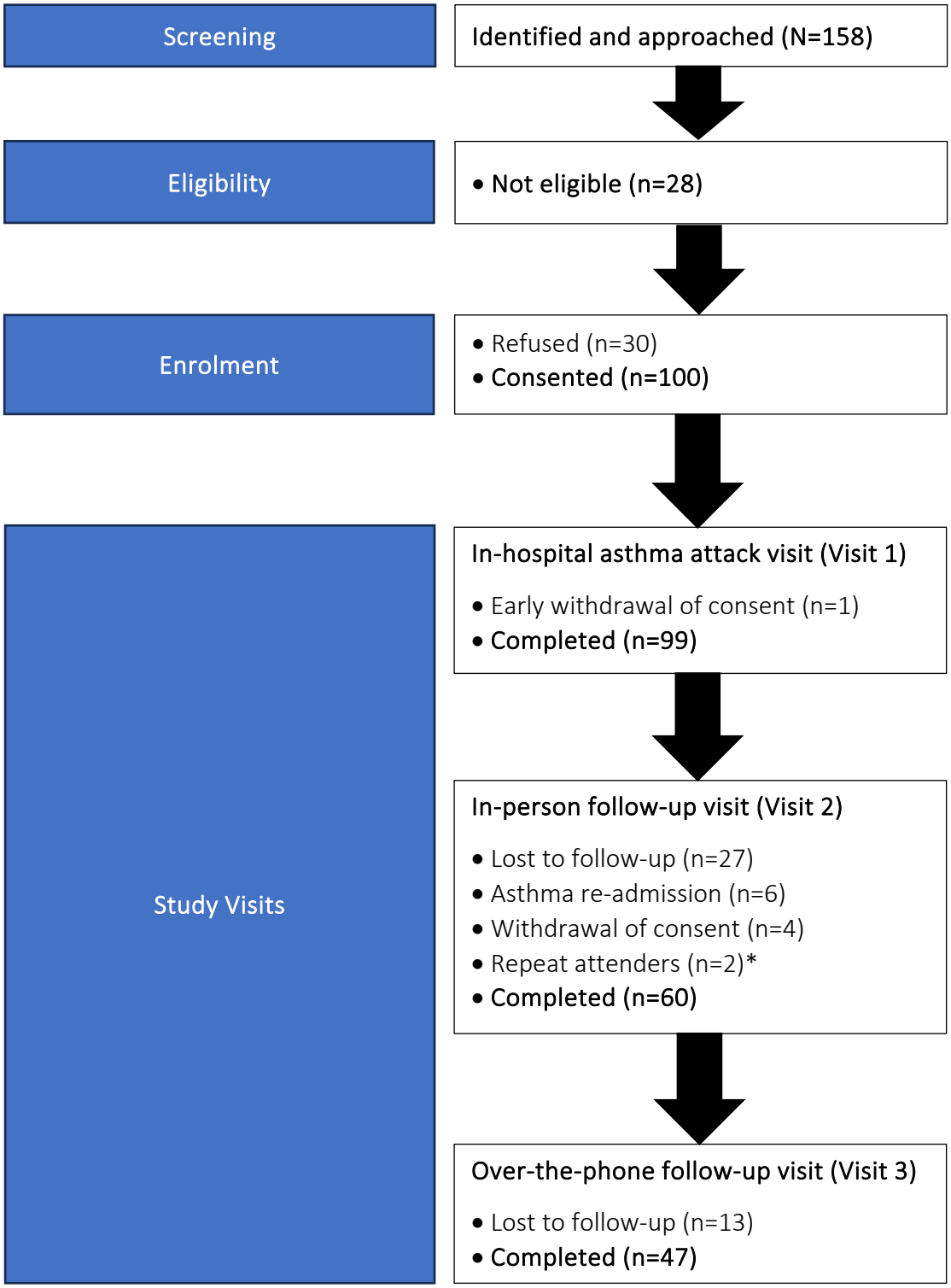
In-person follow-up visit (visit 2)

Of the 99 patients who completed V1, 60 completed visit 2 (V2). 39 patients did not complete V2; of those, 27 were unable to be contacted despite several attempts, 6 were re-admitted for asthma before the follow-up visit and hence were excluded from V2, 4 patients withdrew their consent prior to V2, and 2 repeat attendees were excluded from the study after V1.

Over-the-phone follow-up visit (visit 3)

Of those who completed V2 (n=60), 47 completed the over-the-phone follow-up, visit 3 (V3).

Figure 16. Flowchart of patients screened, identified, excluded, and recruited for the study.



**Two participants were recruited twice for the study. Only the first study participation was considered. The second study participation was removed for both patients.*

Patients' demographics and medical history

The majority of the patients identified were white and female with a mean age of 43.9 years (Table 19). A summary of missing data is presented in Appendix E.

Table 19. Demographic characteristics.

Demographics	N= 97 (100%)
	N (%) unless stated otherwise
Age (Mean ± SD) (years)	43.9 ± 17.0
Gender	
• *Male*	32 (33.0)
• *Female*	65 (67.0)
Ethnicity	
• *White/Caucasian*	69 (71.1)
• *Black/Black British*	10 (10.3)
• *Asian/Asian British*	13 (13.4)
• *Other*	5 (5.2)

89.7% of the patients recruited into the study had a prior diagnosis of asthma. Moreover, 44.3% had atopic comorbidities and 72.2% had significant comorbidities. Medical history and comorbidities are shown in Table 20. 5 (5.2%) of the patients had diagnoses of "asthma mimics" defined as inducible laryngeal obstruction (ILO), dysfunctional breathing, and excessive dynamic airway collapse (EDAC).

Table 20. Medical history and comorbidities.

Medical history and comorbidities	N= 97 (100%)
	N (%)
Previously diagnosed asthma	87 (89.7)
Atopic comorbidities	43 (44.3)
• *Eczema*	23 (23.7)
• *Rhinitis*	33 (34.0)
Significant comorbidities	70 (72.2)
• *Depression*	40 (41.2)
• *Anxiety*	36 (37.1)
• *Nasal polyps*	5 (5.2)
• *Gastroesophageal Reflux Disease (GERD)*	25 (25.8)
• *Hypertension*	15 (15.5)
• *Obesity (BMI ≥30)*	45 (46.4)
Airway diseases	8 (8.3)
• *Chronic obstructive pulmonary disease (COPD)*	1 (1.0)
• *Obstructive sleep apnoea (OSA)*	6 (6.2)
• *Excessive Dynamic Airway Collapse (EDAC)*	1 (1.0)
Asthma Mimics	5 (5.2)
• *Inducible laryngeal obstruction (ILO)*	3 (3.1)
• *Dysfunctional breathing*	1 (1.0)
• *Excessive Dynamic Airway Collapse (EDAC)*	1 (1.0)
Smoking history	
• *Never*	53 (54.6)
• *Current*	19 (19.6)
• *Ex smoker*	25 (25.8)
Latex/alcohol allergy	5 (5.2)
Medication allergies	36 (37.1)

Asthma-specific history

A detailed asthma history collected from patients with previously diagnosed asthma is shown in Table 21. 80.5% of the patients identified reported perceived allergies. Although about 50% indicated knowing how to respond to a low peak flow level, only 29% had an asthma management plan.

Table 21. Asthma specific history.

Previously diagnosed asthma	**n= 87**
Asthma-specific history	**N (%) unless stated otherwise**
Age onset (in years) Median (IQR)	10 (IQR= 0 – 28.5)
Years with asthma (Mean ± SD)	24.8 ± 14.9
Family history of asthma	41 (47.7)
Perceived allergies	70 (80.5)
• *Animals (Cats, Dogs)*	37 (42.5)
• *Inhaled allergens (pollen, house dust mites)*	68 (78.2)
• *Grass, Trees*	40 (45.9)
• *Other: Mold, Seafood, Nuts*	4 (4.6)
• Aspirin allergy	16 (18.4)
Has asthma action plan	26 (29.9)
Has peak flow meter	60 (69.8)
Knows how to respond to a low PEFR	42 (48.8)
Pet ownership	37 (42.5)

PEFR: peak expiratory flow rate.

Asthma medications

Of the 87 patients with previously diagnosed asthma, 10 (11.5%) had no asthma medications at the time of study recruitment, and 5 (5.8%) had short-acting beta-agonists (SABA) only at the time of study recruitment. 7 (8.1%) were on an asthma biological treatment or low-dose OCS at the time of study recruitment (Table 22).

Table 22. Asthma treatment.

Previously diagnosed asthma	**n= 87**
Asthma Treatment	**N (%)**
Not on asthma treatment	10 (11.5)
SABA only	5 (5.8)
On GINA treatment Step	72 (82.8)
• *Step 1*	2 of 72 (2.8)
• *Step 2*	5 of 72 (6.9)
• *Step 3*	11 of 72 (15.3)
• *Step 4*	31 of 72 (43.1)
• *Step 5*	23 of 72 (31.9)
Biologics	5 (5.8)
Low-dose maintenance OCS	2 (2.3)
Biologics or Low-dose maintenance OCS	7 (8.1)

SABA: Short-Acting Beta-Agonists. GINA: Global initiative for asthma. OCS: Oral corticosteroids.

Asthma attack history

44 (50.6%) of the patients with previously diagnosed asthma had >1 OCS courses in the 12 months prior to study recruitment. Details of previous attacks, asthma-related hospital admissions, GP visits, and OCS use are shown in Table 23. Of the 87 patients with a previous asthma diagnosis, 61 reported having at least one asthma-related hospital admission in the past, of whom 15 (24.6%) had >1 admission for asthma in the 12 months prior to study recruitment.

Table 23. Asthma attack history.

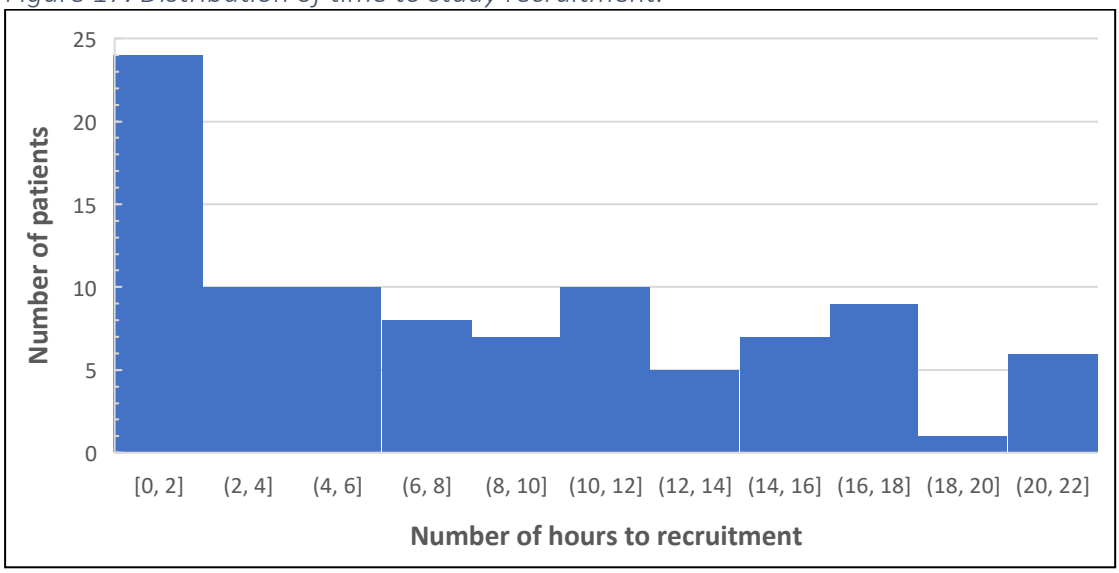
Previously diagnosed asthma	**n= 87**
Asthma attack history	**N (%) unless stated otherwise**
Number of unscheduled visits (A&E, GP, etc) last 12 months Median (IQR)	2 (0 – 5)
>1 unscheduled visits (A&E, GP, etc) last 12 months	50 (57.5)
Number of OCS courses last 12 months Median (IQR)	2 (0 – 4)
>1 OCS courses last 12 months	44 (50.6)
Number of AAs last 12 months Median (IQR)	2 (0 – 4.5)
>1 AAs last 12 months	50 (57.5)
Previous admissions for asthma (ever)	61 (70.1)
• *Number of previous admissions (ever)* Median (IQR)	3 (1 – 8)
• *Number of NON-ITU admissions* last 12 months Median (IQR)	1 (0 – 1)
• *>1 NON-ITU admissions* last 12 months	15 of 61 (24.6)
• *Previous ITU admission (ever)*	14 of 61 (22.9)
• *Previously Intubated (ever)*	5 of 61 (8.2)

OCS: Oral corticosteroids. ITU: Intensive Treatment Unit.

Admission characteristics (visit 1)

Participants were recruited as soon as possible following hospital admission. The median length of time from hospital admission to study recruitment was 7 hours (IQR= 2 – 12.9 hours) (Figure 17) (Table 24).

Figure 17. Distribution of time to study recruitment.



The median length of hospital stay was 2 days (IQR= 1 – 4 days). 9 (9.3%) of the asthma attack admissions had primary discharge diagnoses other than asthma attack recorded on discharge, while 11 (11.3%) had secondary diagnoses documented in their discharge summaries (Table 24). 19 (19.6%) of the patients identified had been treated with oral corticosteroids prior to being admitted, and 87 (89.7%) had received nebulisation prior to study recruitment. 25 (25.8%) of the patients identified were on systemic anti-inflammatory treatment (low-dose maintenance OCS, biologics, or pre-admission treatment with OCS) at the time of study recruitment (Table 24). A summary of missing data is presented in Appendix E.

Table 24. Characteristics of admissions identified.

Admission characteristics	N= 97 (100%)
	N (%) unless stated otherwise
Time to recruitment in hours Median (IQR)	7 (2 – 12.9)
Length of stay in days Median (IQR)	2 (1 – 4)
Different primary discharge diagnosis*	9 (9.3)
Secondary diagnosis with potential relevance to presenting symptoms**	11 (11.3)
Pre-admission treatment with oral corticosteroids	19 (19.6)
Pre-recruitment nebuliser	87 (89.7)
On systemic anti-inflammatory at the time of study recruitment†	25 (25.8)

**Lower respiratory tract infections, inducible laryngeal obstruction, community-acquired pneumonia, influenza A infection, viral bronchitis, eosinophilic pneumonia. **Long covid, viral induced wheeze, type 2 respiratory failure, infective exacerbation of asthma, undiagnosed COPD, an element of upper airway wheeze and anxiety, congestive cardiac failure/obesity hyperventilation syndrome, anxiety/hyperventilation, upper airway noise, possible dysfunctional breathing, multifactorial shortness of breath. †Low-dose OCS, biologics, or pre-admission treatment with OCS.*

Clinical investigations

Pulmonary traits

Physiological parameters

Lung function measures

85 (87.6%) patients successfully completed spirometry as per ATS/ERS guidelines[532]. Airflow limitation (FEV_1/FVC <0.7) was noted in 48 (56.5%) patients (Table 25). The mean lung function ratio was 0.68 (SD= 0.14). There was no difference in the mean lung function ratio between those with (n= 18) and without (n= 67) pre-admission OCS treatment (0.68 vs. 0.68, P= 0.93). Similarly, there was no difference in the mean lung function ratio between those with (n= 10) and without (n= 75) pre-recruitment nebulisation (0.68 vs. 0.68, P= 0.97). Mean Forced Expiratory Flow 25-75% ($FEF_{25-75\%}$) was 48.6% (SD= 29.4%). 58 (68.2%) of those who completed spirometry had $FEF_{25-75\%}$ less than 65% of predicted.

There was no significant correlation between the time spent in the hospital before study recruitment and lung function ratio (rho: 0.07, P= 0.53). Additionally, the

correlation between time to recruitment and FEV_1% and FVC% was also not significant (rho: 0.02, P= 0.81, and rho: -0.006, P= 0.95, respectively).

All patients performed PEFR on admission (N= 97). The mean PEFR% was 76.7% (SD= 24.1%). 44 (45.4%) patients had PEFR limitation (<75%); PEFR limitation was based on patient's best PEFR value in 54 (55.7%) admission and on predicted PEFR value in 43 (44.3%) admission. There was no difference in the mean PEFR% in patients with and without pre-admission OCS and pre-recruitment nebulisation (P= 0.51, P= 0.71, respectively). There was a weak positive correlation between time to study recruitment and on admission PEFR% (rho: 0.27, P= 0.006). A summary of missing data is presented in Appendix E.

Forced oscillation was completed by 94 (96.9%) patients, of whom 48 (51.1%) had increased airway resistance (R5 >5 $cmH_2O/L/s$) as per Lipworth's criteria for defining airflow obstruction[549] (Table 25). The mean R5 value was 5.5 $cmH_2O/L/s$ (SD= 2.3 $cmH_2O/L/s$). There was no difference in the mean R5 in patients with (n= 17) and without (n= 77) pre-admission OCS (P= 0.18). Similarly, there was no difference in mean R5 in those with (n= 8) vs without (n= 86) pre-recruitment nebulisation (P= 0.45). The correlation between R5 and time to recruitment was not significant (rho: -0.05, P= 0.56). The mean R5-20 value was 1.7 $cmH_2O/L/s$. R5-20 was greater than 1 $cmH_2O/L/s$ in 58 (61.7%) of the patients who completed FOT.

In those who completed both spirometry and FOT (n= 83), 8 (9.6%) had the small airways asthma phenotype, defined as per Lipworth's criteria: preserved FEV_1 (>80%) and (FEF_{25-75} <60% OR R5-20 >1 $cmH_2O/L/s$)[112].

Breath sounds

Breath sounds were assessed and documented by a respiratory consultant for all the admissions. 43 (44.3%) of the patients had normal breath sounds on admission, and 52 (53.6%) had wheeze, of whom 7 (13.5%) had expiratory wheeze and 45 (86.5%) had unspecified wheeze. 2 (2.1%) of the admissions had other breath sounds documented, e.g. crackles, crepitations.

Overall objective evidence of airflow limitation

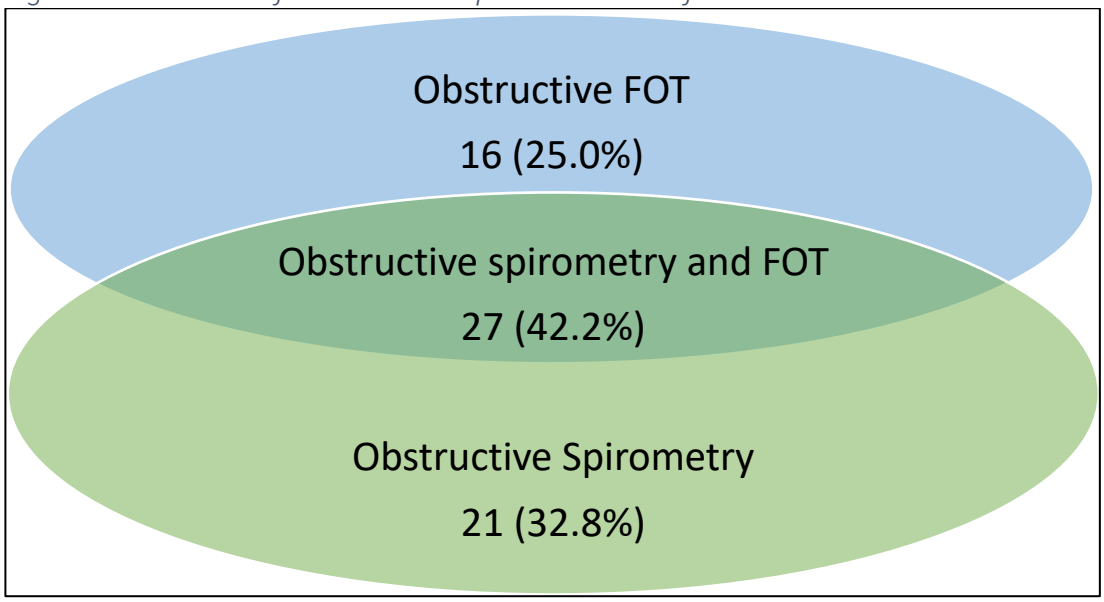
Overall, in 83 participants who completed both objective measures of airflow assessment (spirometry and FOT), 64 (77.1%) had evidence of obstruction on one or both of the measures (Table 25). Of those with evidence of airflow obstruction (n=64), 27 (42.2%) had evidence of obstruction on both measures, 21 (32.8%) had normal FOT but obstructive spirometry, and 16 (25%) had normal spirometry but obstructive FOT (Figure 18). 19 (22.9%) participants had no evidence of obstruction on either measure.

Table 25. Evidence of airflow limitation and small airway impairment.

Lung function measures	Obstructive	Non-obstructive
	N (%)	
Spirometry (FEV_1/FVC <0.7) (n= 85)	48 (56.5)	37 (43.5)
FOT (R5 >5 cmH_2O/L/s) (n= 94)	48 (51.1)	46 (48.9)
FEV_1/FVC <0.7 and/or R5 >5 cmH_2O (n= 83)*	64 (77.1)	19 (22.9)
PEFR (<75%) (n= 97)	44 (45.4)	53 (54.6)
$FEF_{25-75\%}$ (<65%) (n= 85)	58 (68.2)	27 (31.8)
R5-R20 (>1 cmH_2O/L/s) (n= 94)	58 (61.7)	36 (38.3)

**In those who completed both measures (spirometry and FOT, n= 83). FOT: Forced oscillation technique.*

Figure 18. Evidence of obstruction in patients with airflow limitation.



In those who completed both measures (spirometry and FOT, n= 83).

APEX3 vs. the standard of care measure of airflow obstruction

A receiver operating characteristic (ROC) curve analysis of the standard of care measure of airflow obstruction (PEFR) in distinguishing airflow obstruction defined using APEX3

criteria (spirometry FEV_1/FVC <0.7 and/or FOT R5 >5 cmH_2O/L/s) indicated an optimal PEFR level of 81% with a sensitivity of 59% and specificity of 95% (AUC= 0.77, P= <0.001, PPV= 97.5%, NPV= 40.9%); using the GINA 2024 PEFR cutoff (80%) resulted in a sensitivity of 56% and a specificity of 95% (PPV= 97.4%, NPV= 39.2%). A cutoff of 75%, as used in the audit, based on the 2019 BTS guideline, yielded a sensitivity of 50% and a specificity of 95% (PPV= 97.1%, NPV= 36.2%).

In a multivariate logistic model, both PEFR ≤75% (P= 0.01) and wheezing (P= 0.02) were identified as independent predictors of airflow obstruction defined using APEX3 methodology (Table 26).

Table 26. Multivariate logistic regression model analysing factors predicting airflow obstruction as defined using APEX3 methodology.

	Unadjusted OR	95% CI	P value	Adjusted OR	95% CI	P value
BEC ≥0.4 x10^9/L	1.8	0.5 – 8.7	0.37			
FeNO ≥25 ppb	1.1	0.4 – 3.3	0.79			
FeNO >50 ppb	1.8	0.6 – 7.1	0.33			
PEFR <75%	18.0	3.4 – 333.5	0.006	15.4	2.8 – 289.1	0.01
Wheezing	4.7	1.7 – 14.2	0.005	4.5	1.3 – 18.3	0.02
Age (per year increase)	1.4	0.45 – 4.0	0.56			
Gender (females vs males)	1.43	0.53 – 3.7	0.47			
AAQLQ score (per unit increase)	0.9	0.66 – 1.4	0.80			
OCS for asthma in the last 12 months	0.9	0.81 – 1.0	0.09			

BEC: blood eosinophil count. FeNO: fractional exhaled nitric oxide. PEFR: peak expiratory flow rate. FOT: Forced oscillation. AAQLQ: Acute asthma quality of life questionnaire. OCS: Oral corticosteroid.

Inflammatory profile

Blood eosinophil count

Blood differential count was assessed in 95 (97.9%) of the admissions. The median BEC was 0.13 x10^9/L (IQR= 0.04 – 0.41). Median BEC was lower in those on systemic anti-inflammatory treatment at the time of study recruitment compared to those without (0.01 x10^^{9}/L vs. 0.22 x10^^{9}/L, P= <0.001). 24 (25.3%) patients had a BEC ≥0.4 x10^9/L, while 45 (47.4%) had a BEC ≥0.15 x10^9/L (Table 27). A summary of missing data is presented in Appendix E.

Fractional exhaled nitric oxide

91 (93.8%) of the patients successfully completed FeNO measurement. The median FeNO value was 26 ppb (IQR= 11.5 – 59.5). FeNO was significantly lower in those on systemic anti-inflammatory treatment at the time of study recruitment (14 ppb vs. 30.5 ppb, P= 0.02). FeNO was ≥25 ppb in 46 (50.6%) of the patients and ≥20 ppb in 55 (60.4%) of the patients (Table 27).

Sputum eosinophil count

Sputum was spontaneously obtained in 68 patients and induced in 6 patients. 52 of the sputum samples were successfully processed for differential cell count. 16 (30.8%) of the samples had a SEC ≥3%, while 20 (38.5%) had a SEC ≥2% (Table 27). The median sputum eosinophil percentage was 0.25% (IQR= 0 – 3.9). Median SEC was similar in those with and without systemic anti-inflammatory treatment at the time of study recruitment (0.25% vs. 0.38%, P= 0.20).

T2-high inflammation

55 (56.7%) of the patients had evidence of T2-high inflammation as determined using APEX3 criteria ($T2^{APEX3}$) (BEC ≥0.4 x10^9/L, FeNO ≥25 ppb, or SEC ≥3%). This number was much higher when using GINA criteria for persistent T2-high inflammation ($T2^{GINA}$) (BEC ≥0.15 x10^9/L, FeNO ≥20 ppb, or SEC ≥2%) (Table 27).

Table 27. T2-high inflammatory biomarkers in patients recruited.

Inflammatory measures	Raised	Normal
	N (%)	
$T2^{APEX3}$ (n= 97)	55 (56.7)	42 (43.3)
• BEC ≥0.4 $x10^9$/L	24 of 95 (25.3)	71 of 95 (74.7)
• FeNO ≥25 ppb	46 of 91 (50.6)	45 of 91 (49.4)
• SEC ≥3%	16 of 52 (30.8)	36 of 52 (69.2)
$T2^{GINA}$ (n= 97)	72 (74.2)	25 (25.8)
• BEC ≥0.15 $x10^9$/L	45 of 95 (47.4)	50 of 95 (52.6)
• FeNO ≥20 ppb	55 of 91 (60.4)	36 of 91 (39.6)
• SEC ≥2%	20 of 52 (38.5)	32 of 52 (61.5)

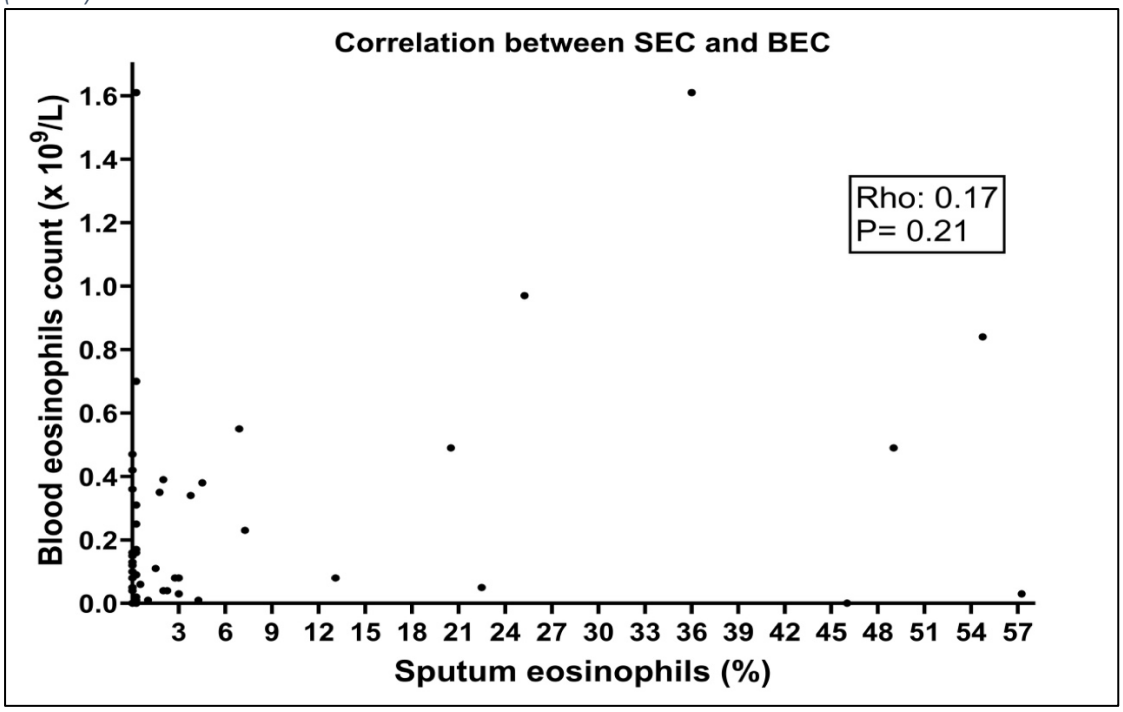
BEC: Blood eosinophil count. FeNO: fractional exhaled nitric oxide. SEC: Sputum eosinophils count.

Association between T2-high parameters

Correlation between sputum eosinophils and blood eosinophils

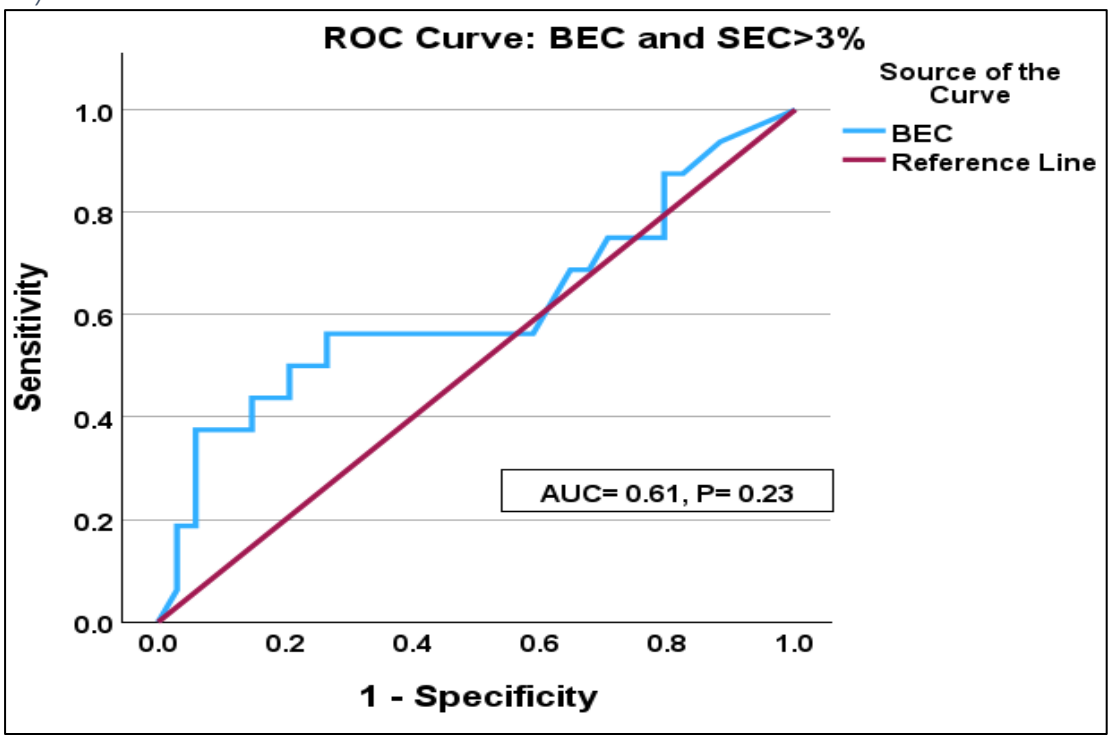
In those with both blood and sputum samples obtained (n= 50), there was no significant linear correlation between sputum eosinophils percent and blood eosinophils count (Rho: 0.17, P= 0.21) (Figure 19). A BEC of 0.48 $x10^9$/L was found to best identify SEC ≥3% with a sensitivity of 38% and specificity of 94% (AUC= 0.61, P= 0.23; PPV= 73.8%, NPV= 77.3%; Figure 20). Similar numbers were observed when only including those not on systemic anti-inflammatory treatment at the time of study recruitment (n= 36) (AUC= 0.61, P= 0.31; optimal cutoff= 0.48 $x10^9$/L, sensitivity= 43%, specificity= 91%, PPV= 68.0%, NPV= 78.2%; Rho= 0.14, P= 0.42).

Figure 19. Relationship between blood eosinophils count and sputum eosinophil% in all patients including those on systemic anti-inflammatory at the time of study recruitment (n= 50).



SEC%: sputum eosinophil%. BEC: blood eosinophils count.

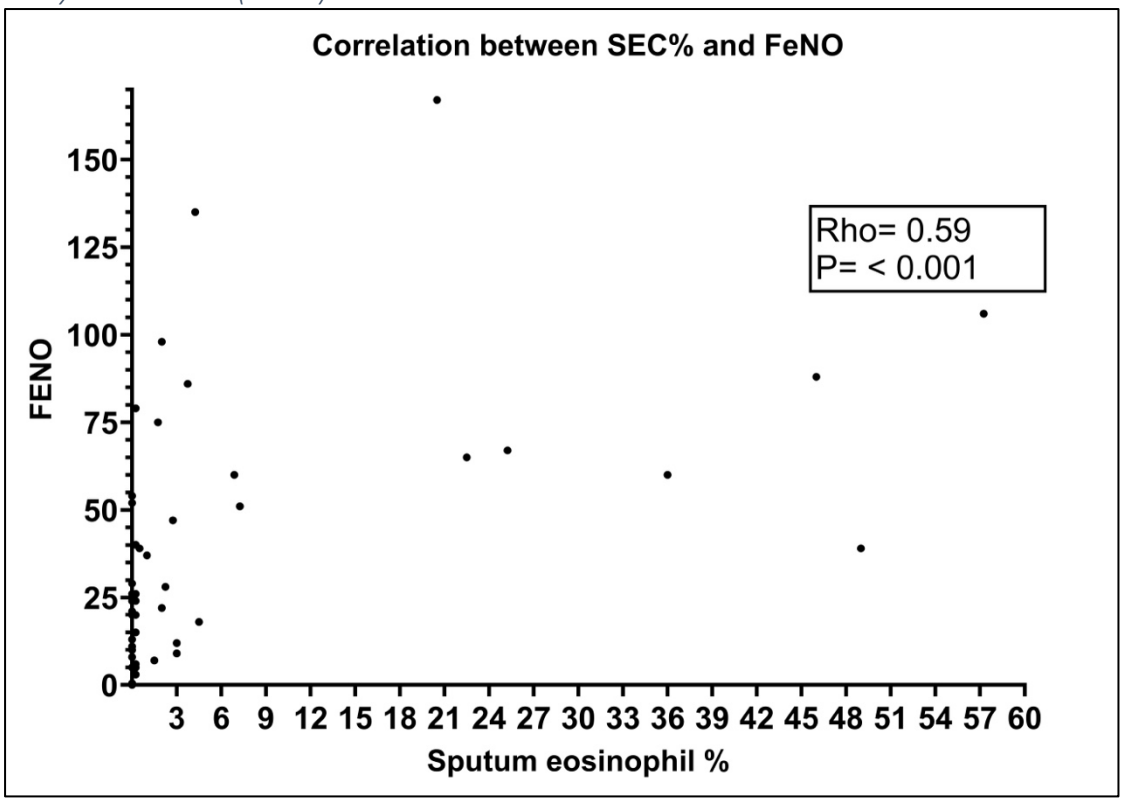
Figure 20. Receiver-operating characteristic curve to determine the optimal blood eosinophils count threshold that identifies sputum eosinophil% ≥3% in all patients including those on anti-inflammatory treatment at the time of study recruitment (n= 50).



Correlation between sputum eosinophils and FeNO

In those with both FeNO and sputum samples obtained (n= 48), a significant, moderate correlation was noted between sputum eosinophils percent and FeNO (Rho: 0.59, P <0.001) (Figure 21). A FeNO cutoff of 49 ppb identified SEC ≥3% with a sensitivity of 71% and a specificity of 85% (AUC= 0.81, P= 0.001, PPV= 66.1%, NPV= 87.7%) (Figure 22). When only including those not on systemic anti-inflammatory treatment at the time of study recruitment (n= 35), FeNO still moderately correlated with SEC (Rho= 0.53, P= 0.001); a FeNO threshold of 57 ppb identified SEC ≥3% with a sensitivity of 58% and a specificity of 96% (AUC= 0.78, P= 0.002, PPV= 86.6%, NPV= 83.7%). The AUC for FeNO in identifying SEC ≥3% was higher than that of BEC, but this was not significantly different (P= 0.11; Figure 23).

Figure 21. Relationship between fractional exhaled nitric oxide and sputum eosinophil% in all patients including those on systemic anti-inflammatory treatment at the time of study recruitment (n= 48).



SEC%: sputum eosinophils %.

Figure 22. Receiver-operating characteristic curve to determine the optimal fractional exhaled nitric oxide threshold that identifies sputum eosinophil ≥3% in all patients including those on systemic anti-inflammatory treatment at the time of study recruitment (n= 48).

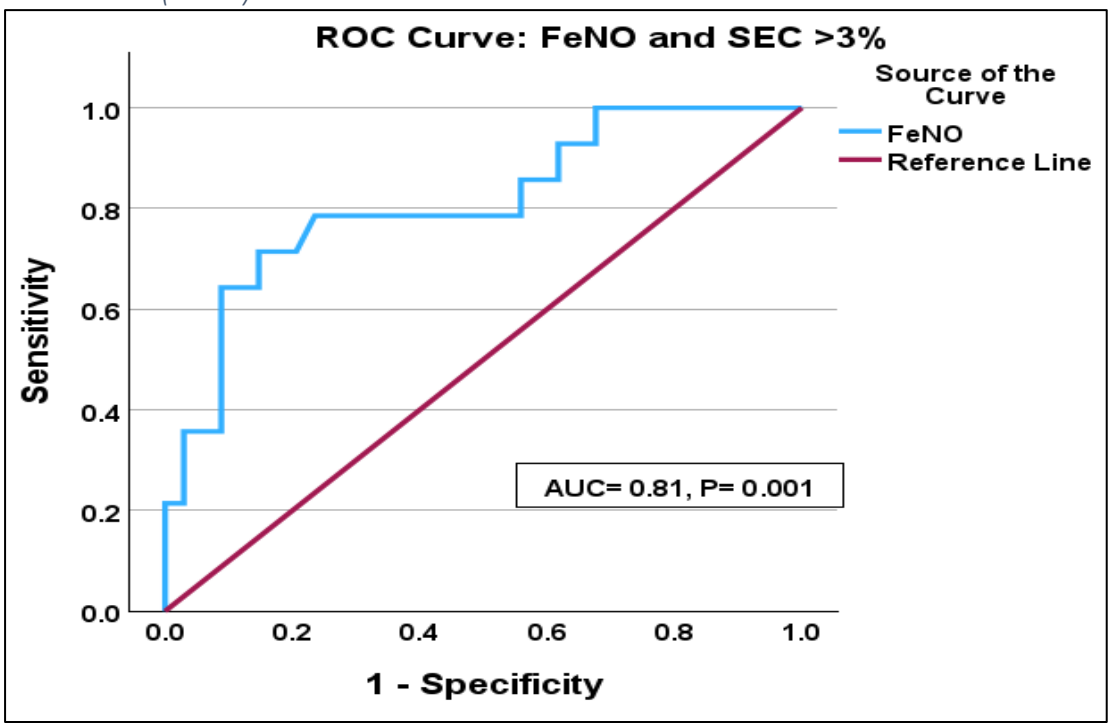
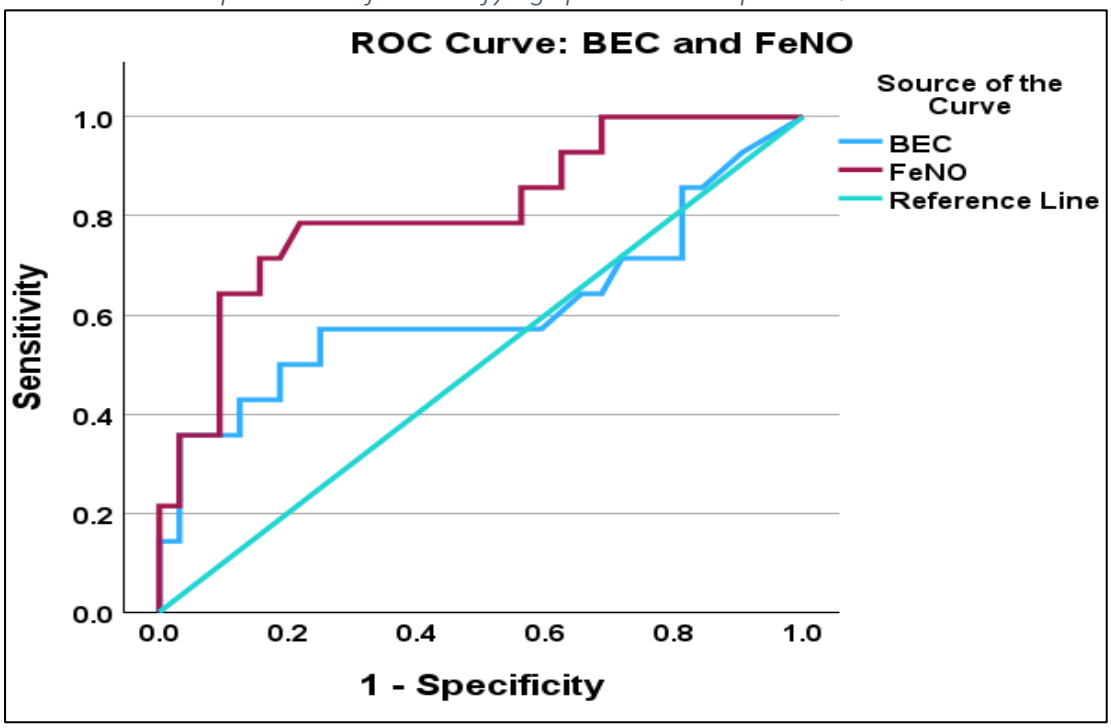


Figure 23. Comparing Receiver-operating characteristic curves of exhaled nitric oxide and blood eosinophils count for identifying sputum eosinophils ≥3%.



There was a significant association between SEC ≥3% and BEC ≥0.4 x10^9/L (OR= 4.5; CI= 1.1 to 20.9; P= 0.04). There was no association between BEC and the odds of a SEC ≥3% (OR= 4.3, CI= 0.8 to 33.0, P= 0.10).

A significant association was noted between FeNO ≥25 ppb and SEC ≥3% (OR= 5.9; CI= 1.5 to 30.0; P= 0.02). Similarly, there was a significant association between SEC ≥3% and FeNO levels ≥50 ppb (OR= 14.5; CI= 3.5 to 73.4; P= <0.001). The odds of a SEC ≥3% increases by 4% for each unit increase in FeNO level (OR= 1.04, CI= 1.02 to 1.07, P= 0.002). A multivariate regression model analysis indicated that FeNO ≥50 ppb was the only predictor of SEC ≥3% (Table 28).

A significant association was noted between FeNO ≥25 ppb and BEC above ≥0.4 x10^9/L (combined) and SEC ≥3% (OR= 7.7, CI= 1.4 to 59.6, P= 0.02).

Table 28. A logistic regression model identifying predictors of SEC ≥3%.

	Unadjusted OR	95% CI	P value	Adjusted OR	95% CI	P value
BEC ≥0.4 x10^9/L	4.5	1.1 – 20.9	**0.04**			
FeNO ≥25 ppb	5.9	1.5 – 30.0	**0.02**			
FeNO >50 ppb	14.5	3.5 – 73.4	**<0.001**	14.5	3.5 – 73.4	**<0.001**
PEFR <75%	6	1.7 – 25.3	**0.008**			
FOT R5 ≥5 $cmH_2O/L/s$	1.2	0.35 – 3.9	0.81			
FEV_1/FVC <0.7	5.1	1.3 – 25.9	**0.02**			
Wheezing	1.5	0.5 – 5.2	0.51			
Age (per year increase)	0.99	0.96 – 1.03	0.94			
Gender (females vs males)	0.7	0.20 – 2.8	0.64			
AAQLQ score (per unit increase)	1.6	1.0 – 2.5	**0.03**			
OCS for asthma in the last 12 months	0.7	0.4 – 0.9	**0.04**			

BEC: blood eosinophil count. FeNO: fractional exhaled nitric oxide. PEFR: peak expiratory flow rate. FOT: Forced oscillation technique. AAQLQ: Acute asthma quality of life questionnaire. OCS: Oral corticosteroids.

Sputum neutrophil count

34 (65.4%) of the sputum samples had a SNC ≥65%. The median SNC percentage was 87.4% (IQR= 54.6 – 97.3). Median SNC was not different between those with and without pre-admission OCS treatment (95.8% vs. 82.8%, P= 0.14).

Overall inflammation

68 (70.1%) of the patients had evidence of raised $T2^{APEX3}$/neutrophilic inflammation compared to 81 (83.5%) when using $T2^{GINA}$/neutrophilic criteria (Table 29).

34 (35.1%) of the patients had evidence of $T2^{APEX3}$ inflammation (BEC ≥0.4, or FeNO ≥25 ppb, or SEC ≥3%) without sputum neutrophilia (neutrophils <65%). 13 (13.4%) of the patients had sputum neutrophilia (neutrophils ≥65%) without evidence of $T2^{APEX3}$ inflammation. 21 (21.6%) had increased markers of $T2^{APEX3}$ inflammation along with increased SNC percentage, and 29 (29.9%) patients had neither eosinophilic nor neutrophilic inflammation (Figure 24 and Figure 25).

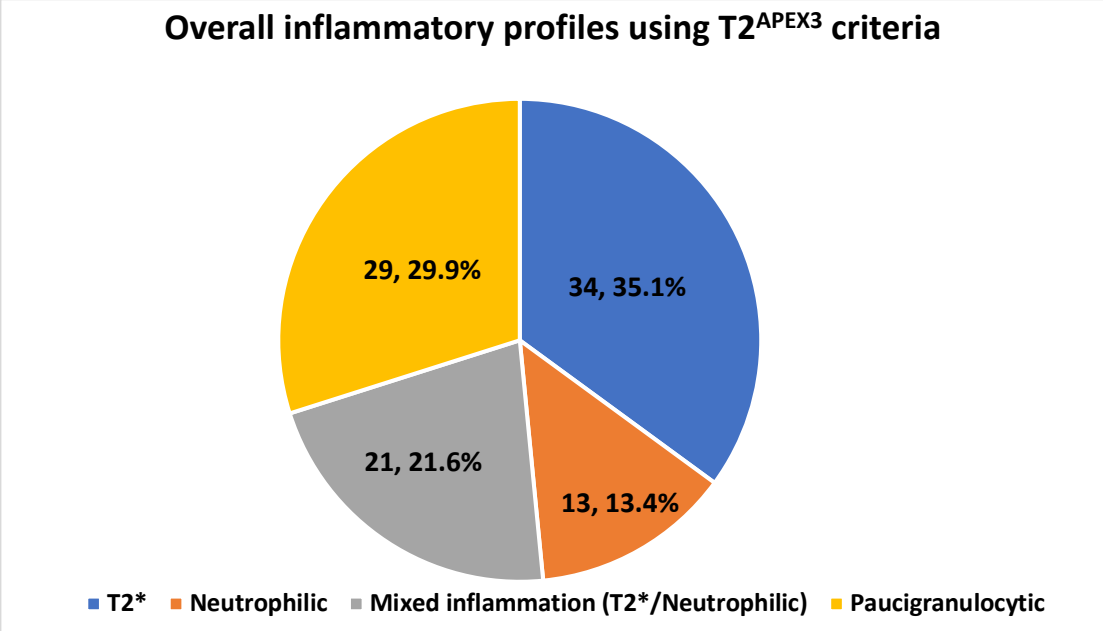
Using GINA criteria, 47 (48.5%) of the patients had T2-high inflammation, 9 (9.3%) had neutrophilic inflammation, 25 (25.8%) had a mixed profile and 16 (16.5%) had a paucigranulocytic profile (Figure 26).

Table 29. Overall Inflammatory biomarkers in patients recruited.

Inflammatory measures	Raised	Normal
	N (%)	
Neutrophilic (n= 52)*	34 (65.4)	18 (34.6)
$T2^{APEX3}$ (n= 97)	55 (56.7)	42 (43.3)
$T2^{APEX3}$/Neutrophils (n= 97)	68 (70.1)	29 (29.9)
$T2^{GINA}$ (n= 97)	72 (74.2)	25 (25.8)
$T2^{GINA}$/Neutrophils (n= 97)	81 (83.5)	16 (16.5)

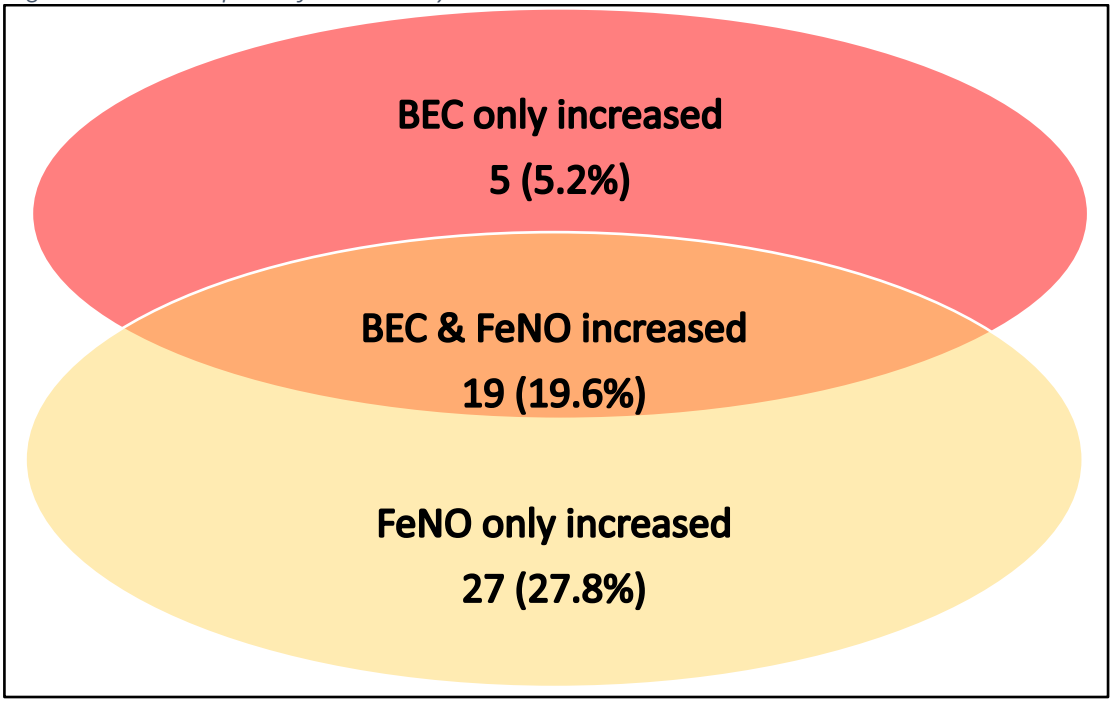
**In those with sputum differential cell count results available (n= 52).*

Figure 24. Inflammatory profiles in patients recruited ($T2^{APEX3}$).



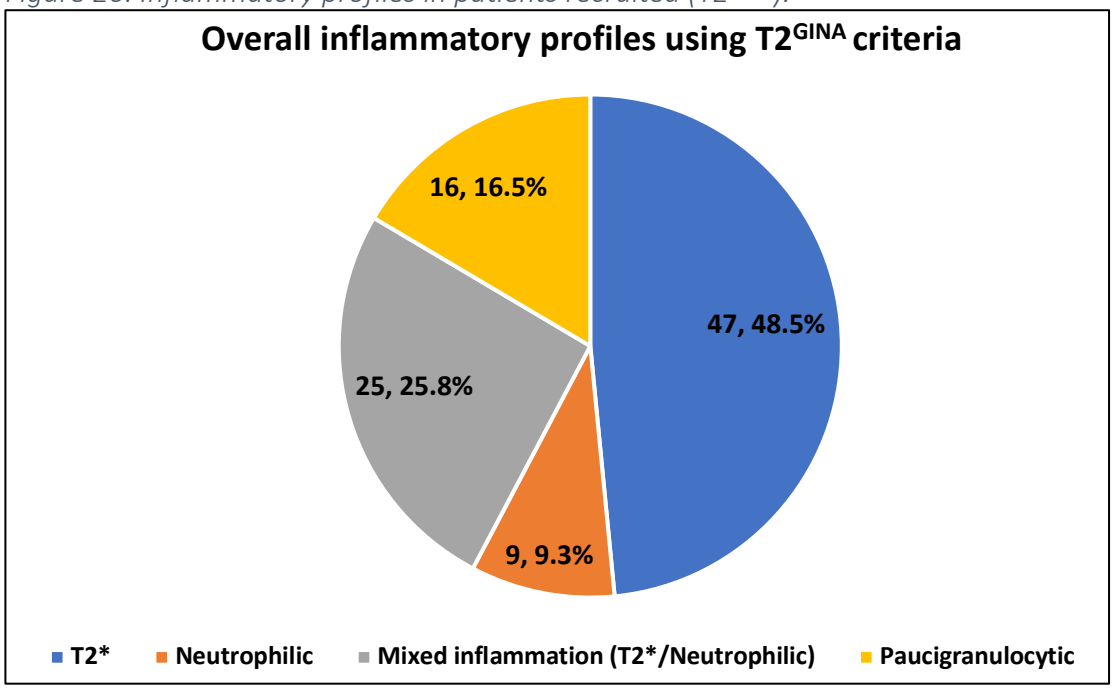
**Using $T2^{APEX3}$ criteria.*

Figure 25. Overlap in inflammatory markers.



Using APEX3 criteria.

Figure 26. Inflammatory profiles in patients recruited ($T2^{GINA}$).



**Using $T2^{GINA}$ criteria.*

Cytokine profiling

Cytokine profiling was completed in 78 of 97 patients at visit 1. The 13 cytokines measured were Interleukin (IL) 5, IL-13, IL-4, IL-33, Thymic stromal lymphopoietin (TSLP), IL-17, IL-8, IL-1β, IL-6, Tumour Necrosis Factor (TNF) α, Interferon (IFN) α, IFN-γ, and IFN-β (Table 30). A summary of missing data is presented in Appendix E.

Table 30. Cytokines profiling completed on admission.

Cytokine profiles	Median (IQR)
T2-high cytokines	
• IL-5 (n= 37)	2.8 (1.6 – 5.9)
• IL-13 (n= 65)	232.1 (102.0 – 373.1)
• IL-4 (n= 77)	41.8 (32.7 – 57.5)
Epithelial derived cytokines	
• IL-33 (n= 74)	74.1 (49.2 – 113.6)
• TSLP (n= 77)	1.6 (1.1 – 2.3)
Th17 cytokines	
• IL-17 (n= 77)	395.6 (253.1 – 521.8)
Pro-inflammatory cytokines	
• IL-8 (n= 65)	1406.9 (574.9 – 2593.7)
• IL-1β n= 77)	52.6 (23.8 – 105.5)
• IL-6 (n= 77)	19.1 (7.6 – 37.2)
• TNF-α (n= 77)	14.2 (7.3- 28.1)
Interferons	
• IFN-α (n= 77)	10.6 (6.7 – 20.5)
• IFN-γ (n= 76)	4.8 (2.9 – 8.5)
• IFN-β (n= 45)	12.9 (3.7 – 21.9)

Evidence of infection

Sputum microscopy, culture and sensitivity

56 sputum samples were analysed for bacterial culture and sensitivity, of which 5 (8.9%) isolated microorganisms (n=1, Haemophilus Influenzae; n= 2, Moraxella Catarrhalis; n= 1, Neisseria meningitidis; n= 1, Staphylococcus Aureus) (Table 31).

Throat swab

Respiratory virus throat swab was performed in 44 asthma attack admissions and was positive in 10 of the admissions (n=6, Influenza A virus; n= 4, Respiratory syncytial virus) (Table 31).

C-reactive protein

C-reactive protein (CRP) was assessed in 96 admissions and was increased (>10 mg/L) in 35 (36.5%) (Table 31).

Blood neutrophil count

Blood neutrophil count (BNC) was assessed in 95 admissions and was increased (>8 $x10^9$/L) in 30 (31.6%). There was no difference in the BNC between patients with and without pre-admission OCS (P= 0.48).

Overall evidence of infection

Overall, 55 (56.7%) of the patients had investigations consistent with infection (Positive sputum MC&S, PCR throat swab, or increased CRP or BNC) (Table 31).

Table 31. Evidence of infection assessed on admission.

Infection measures	Positive or increased	Normal
	N (%)	
Sputum MC&S (n= 56)	5 (8.9)	51 (91.1)
PCR throat swab (n= 44)	10 (22.7)	34 (77.3)
CRP (>10 mg/L) (n= 96)	35 (36.5)	61 (63.5)
BNC (>8 $x10^9$/L) (n= 95)	30 (31.6)	65 (68.4)
Overall infection status† (n= 97)	55 (56.7)	42 (43.3)

MC&S: Sputum microscopy culture sensitivities. CRP: C-reactive protein. BNC: Blood neutrophil count. †Positive sputum MC&S, PCR throat swab, or increased CRP or BNC.

Symptoms score

The Acute Asthma Quality of Life Questionnaire was completed in 95 admissions. The mean AAQLQ score was 3.9 (SD= 1.46). The mean score was 3.9 (SD= 1.46) for the symptoms domain and 3.8 (SD= 1.67) for the emotional domain.

Extra-pulmonary traits

Upper airway symptoms

Upper airway symptoms, assessed using SNOT-22 questionnaire in 62 of the patients with no diagnosis of rhinitis, were increased in 59 patients (95.2%). 10 of 62 (16.1%) of the patients had mild SNOT-22 score, 24 (38.7%) had moderate score, and 25 (40.3%) had severe score.

Gastroesophageal Reflux Disease

GERDQ was completed by 71 of 72 patients without a formal GERD diagnosis, of whom 8 (11.3%) had an increased GERDQ score, indicating likely undiagnosed GERD.

Vocal cord dysfunction

The Pittsburgh vocal cord dysfunction questionnaire score was increased in 45 (49.5%) of the 91 patients who completed the questionnaire and had no previous VCD diagnosis.

Dysfunctional breathing

Only one patient had a diagnosis of dysfunctional breathing. The Nijmegen Questionnaire for dysfunctional breathing was increased in 36 of the 94 (38.3%) patients who completed the questionnaire.

Anxiety and depression

36 of the patients recruited had a diagnosis of anxiety. In those without anxiety (n= 61), 60 completed the HADS questionnaire, of whom 9 (15%) scored high in the anxiety domain of the questionnaire, suggesting an increased likelihood of anxiety. None of the 56 patients without a diagnosis of depression scored high in the depression domain of the HADS questionnaire.

BMI

45 (46.4%) of the patients had a BMI ≥30, and 72 (74.2%) had a BMI ≥25. The mean BMI was 30.5 (SD= 8).

Vitamin D

Vitamin D was assessed in 95 of the admissions and was insufficient (25-50 nmol/L) in 35 (36.8%) and severely deficient (<25 nmol/L) in 16 (16.8%) of the admissions.

Risk and behavioural traits

Medication adherence report scale

The medication adherence report scale (MARS) was completed by 84 (96.6%) of the 87 patients with previous asthma diagnosis. The median MARS score was 23 (IQR 20 – 25), indicating good adherence.

Medication Possession Ratio

Medication Possession Ratio (MPR%) was collected for 80 (91.9%) of the patients with previously diagnosed asthma. The median MPR was 62.9% (IQR= 14.2 – 100). 41 (51.3%) of the patients with previously diagnosed asthma had a MPR less than 75%.

Inhaler technique

Inhaler technique was assessed using the NHS Liverpool Clinical Commissioning Group-inhaler device checklist in 67 out of 72 patients on regular asthma medications. 33 (49.3%) had good technique, 27 (40.3%) had moderate technique, and 7 (10.5%) had poor technique. A summary of missing data is presented in Appendix E.

Evidence of acute asthma

Lung function measures

In 83 (85.6%) patients who completed both spirometry and FOT, 64 (77.1%) had evidence of airflow limitation (as determined by spirometry (FEV_1/FVC) and/or Forced oscillation (R5)) (Table 25); those will be referred to as *Likely Acute Asthma-Related Admissions* (LAARA, n= 64, 77.1%). 19 of 83 (22.9%) had no evidence of airflow limitation on either spirometry or FOT (Table 25); those will be referred to as *Likely Not Acute-Asthma Admissions* (LINAA, n= 19, 22.9%).

Inflammatory profile in LAARA (n= 64) and LINAA (n= 19)

Using APEX3 criteria for inflammation, the proportion of participants with raised T2-high inflammatory markers (FeNO ≥25 ppb, blood eosinophil count ≥0.4 $x10^9$/L, or sputum eosinophil count ≥3%) was similar in the LAARA compared to the LINAA group (Table 32). The proportion of patients with increased sputum neutrophils count (≥65%) was similar between the groups. Overall, LINAA were less likely to have raised $T2^{APEX3}$ and/or neutrophilic inflammatory markers (52.6% vs. 78.1%, P= 0.03) (Table 32).

Table 32. Evidence of inflammation in admissions with vs. without airflow limitation.

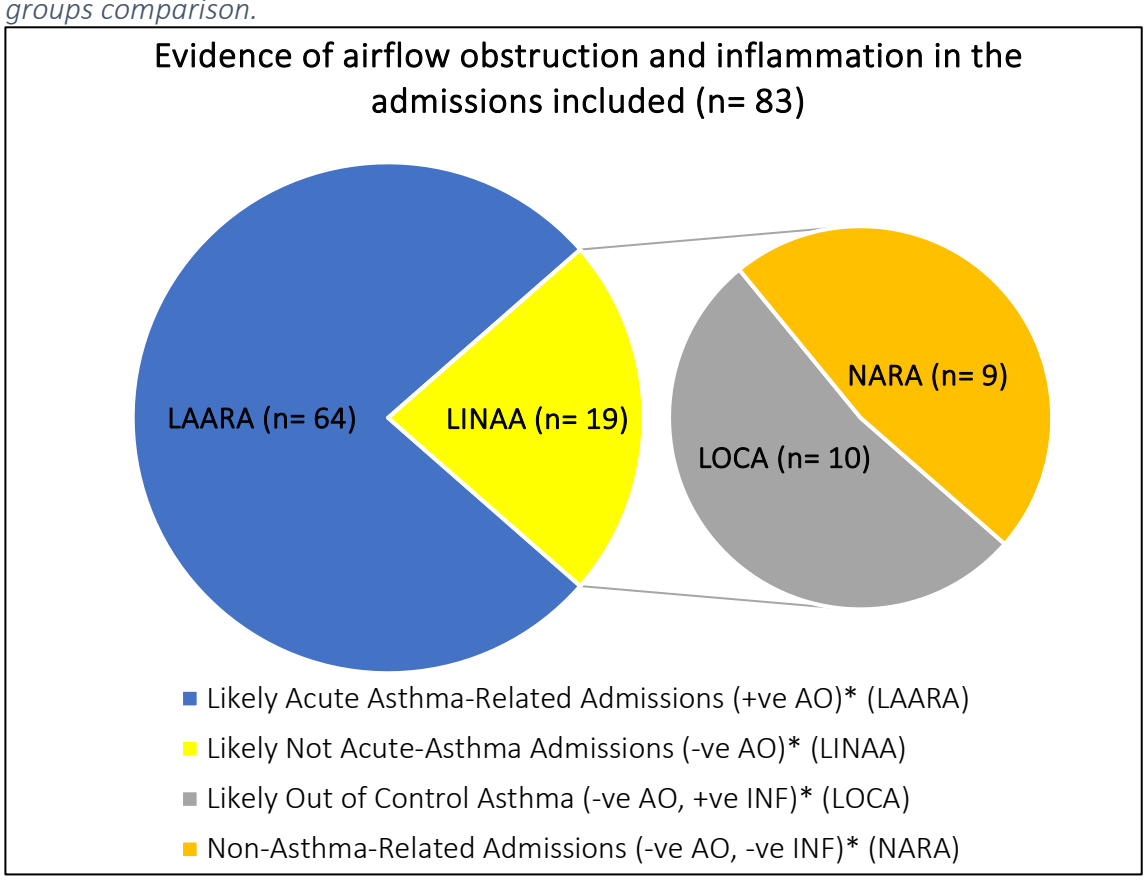
Inflammation	N= 83 (100.0%)	LAARA 64 (77.1%)	LINAA 19 (22.9%)	P value
	N (%)			
Raised $T2^{APEX3}$	47 (56.6)	38 (59.4)	9 (47.4)	0.35
Raised neutrophils*	32 of 45 (71.1)	25 of 35 (71.4)	7 of 10 (70.0)	1
Raised $T2^{APEX3}$/Neutrophils*	60 (72.3)	50 (78.1)	10 (52.6)	0.03
Normal $T2^{APEX3}$/Neutrophils*	23 (27.7)	14 (21.9)	9 (47.4)	

**In those with sputum differential cell count results available.*

10 (52.6%) of the LINAA had raised inflammatory markers ($T2^{APEX3}$/neutrophils) (without airflow limitation) (Table 32) and are likely to be poorly controlled asthma; those will be referred to as *Likely Out of Control Asthma Admissions* (LOCA, n= 10) (Figure 27). 9 (47.4%) of the LINAA had normal inflammatory markers; those had no evidence of airflow limitation, had inflammatory markers ($T2^{APEX3}$/neutrophils) within normal levels (Table 32), and are likely to be non-asthma related and will be referred to as *Non-Asthma-Related Admissions* (NARA, n= 9) (Figure 27).

The following section (Chapter 4) will compare LAARA (n= 64) to LINAA (n= 19) in patients who completed both measures of airflow assessment (n= 83) (Figure 27). The comparison between the subgroups LAARA vs. NARA and LAARA vs. LOCA will be presented in Appendices G and H, respectively.

Figure 27. Categories of asthma attack admissions included in the subsequent, between groups comparison.



*In those who completed both spirometry and FOT (n= 83). *+ve: positive, -ve: negative, AO: airflow obstruction, INF: inflammation.*

Discussion

Findings from this study are consistent with the results of the audit; a significant proportion of patients admitted for AA did not have evidence of airflow obstruction assessed using POC objective measures of lung function. These measures, along with other POC measures of inflammation, were found to be well tolerated, even in very unwell patients. Additionally, this study further confirmed the heterogeneity of acute asthma, which is, as yet, poorly described when compared to more detailed descriptions of the heterogeneity of stable asthma.

Results from this study suggest possible contribution of small airway dysfunction in AAs, in contrast to the notion that asthma attacks are purely episodes of large airway bronchospasm and in line with literature highlighting the crucial role of the small airways in asthma. There was a discrepancy between airflow obstruction detected through oscillometry and spirometry. This inconsistency is not well understood but likely relates to the capabilities of these tests to identify different patterns/locations of airflow abnormalities.

Evidence of airflow obstruction

Supporting the findings from the clinical audit (Chapter 2), ~23% of the 83 patients included in this analysis, as opposed to ~16% in the audit, did not have evidence of airflow obstruction suggestive of acute asthma. This difference may be due to the more robust POC measures of airflow assessment employed during this prospective study. While studies have consistently highlighted that a significant number of the patients labelled with "diagnoses" of asthma do not meet guideline diagnostic criteria[499,500], such misclassification has not been thoroughly investigated in the settings of acute asthma, and studies investigating misdiagnosis of AAs are limited. The study of Centurion and colleagues[493] was the first to objectively assess the frequency of misdiagnosis in patients with AAs admitted to general medicine wards at four university-affiliated medical centres. The authors defined airflow limitation as FEV_1/FVC <lower limit of normal and reported that 17% of those admitted did not have airflow limitation. A slightly higher percentage was identified in the present study (22.9%), which could potentially be explained by the inclusion of another complementary measure of airflow obstruction (FOT), using a fixed cutoff FEV_1/FVC to define airflow

limitation and, most likely, that the assessment of lung function in the present study was performed within hours of hospital admission (median of 7 hours) as compared to a median of 1 day in the study of Centurion and colleagues.

Point of care measures of airflow obstruction and inflammation

Misdiagnosis of AAs is likely to improve through routine use of objective tests of lung function and inflammation; however, these are currently underutilised, perhaps due to concerns about inadequate test quality during acute episodes, limited evidence supporting their utility in this context, and constraints related to capacity and availability of trained staff to administer the tests. The present study found that POC measurement of airflow obstruction using spirometry is well tolerated, even in very unwell patients; adequate quality spirometry was performed in 87.9% of the patients included in the study. This is in line with studies demonstrating the utility of spirometry in the assessment of lung function in patients hospitalised for exacerbation of airway diseases[560], including asthma[502]. The study of Centurion and colleagues[493] demonstrated that 78% of the patients admitted for acute asthma were able to perform spirometry within a median of 1 day following hospital admission. The present study extends these findings by showing that adequate spirometry can be obtained in the vast majority of patients within a few hours following hospitalisation for AAs. Further, as expected, FOT was successfully performed by 97% of the patients, in keeping with studies demonstrating the feasibility of FOT for the assessment of lung function even in infants[561] and acutely ill children[562,563].

Our study found that while 56.5% and 51.1% had obstructive spirometry (FEV_1/FVC) and FOT (R5), respectively, only 45.4% had PEFR limitation (<75%). The sensitivity of PEFR, using various %cutoffs in identifying objectively defined airflow obstruction in the present study was low, which is similar to previously reported figures[564].

Airflow limitation is a major component of the definition and the clinical assessment of AAs, and thus, it would be reasonable to objectively assess the level of lung function impairment during these episodes. However, spirometry is commonly underutilised for the assessment of airflow limitation during acute asthma[565,566], and FOT still remains a research tool. It is likely, and logical that spirometry would be useful during the assessment and management of asthma attacks owing to the limited specificity of

respiratory symptoms and the limited accuracy of clinically established diagnoses[476,567]. Indeed, many diseases, including heart failure[526], ILO[433,568], excessive dynamic airway collapse[569], and breathing pattern disorder[570-572], can present acutely with asthma-like symptoms and are often mistaken for acute asthma. In addition, underutilisation of objective measures of airflow obstruction may also lead to inaccurate assessment and underappreciation of the severity of AAs and subsequent inadequate treatment and delayed referral to specialist centres, which has been linked to poor asthma outcomes, including asthma mortality[510].

Further, there is a well-recognised dissociation between asthma symptoms and the level of lung function decline measured using PEFR and FEV_1[573-575]. Shim and Williams[525] demonstrated that the association between wheezing and the severity of airflow obstruction (assessed using PEFR) in asthma patients may vary greatly. Teeter and colleagues[575] reported that FEV_1 and PEFR varied more than 10% in 64% of the patients. In addition, Aggarwal et al.[576] retrospectively reviewed spirometry reports of more than 6000 patients with obstructive airway diseases and concluded that PEFR and FEV_1 cannot be used interchangeably as PEFR% underestimated FEV_1% in patients with severe obstruction and overestimated FEV_1% in those with less severe obstruction. Such disagreement has also been noted in the settings of acute asthma; although reported correlation coefficients between the two measures range from 0.73 to 0.9[577,578], the correlation tends to decrease with greater airflow impairment[577].

In contrast to the significant discordance between spirometry and FOT identified in the present study, studies have consistently reported a strong correlation between spirometry indices and those of FOT[579-581]. The correlation between R5, a commonly reported measure of airflow obstruction, and PEFR, however, is not clear. Qi and colleagues[581] evaluated the utility of FOT indices in assessing severity of spirometry-defined airflow obstruction in 80 asthma patients and controls and reported a strong correlation between R5 and percent predicted PEFR. Vink et al.[580] assessed the sensitivity and specificity of FOT parameters and PEFR in identifying reduction in FEV_1% following methacholine challenge in children; while R5 and PEFR performed similarly in detecting a 20% fall in FEV_1, R5 was superior to PEFR in detecting smaller changes in FEV_1. Song et al.[582] found no correlation between bronchodilator responses in children (with asthma) aged 3-6 years measured using PEFR and R5.

Our study suggested that POC assessment of inflammatory measures during AAs is feasible. Although 70% of the patients were able to spontaneously produce sputum shortly after admission, which is expected during AAs[583], sputum processing is labour-intensive and time-consuming, and the procedure of sputum induction, in the case patients do not spontaneously produce sputum, is difficult and has potential risks, particularly during acute deterioration of asthma. Although sputum inflammatory cell counts are the gold standard measure of airway inflammation, FeNO is a robust surrogate, which the present study has demonstrated to be highly feasible; 93.8% of the patients in the present study were able to perform FeNO measurement soon after hospital admission, which is in line with previous literature[475,584].

FeNO is a robust proxy of T2-high activity that is regulated by key T2-high cytokines (IL-13, IL-4)[585,586]. FeNO correlates with bronchoalveolar lavage eosinophils[587], bronchial biopsy eosinophils[588], sputum eosinophils[589-591], bronchial hyperresponsiveness[589,590], blood eosinophils and IGE[592], symptoms and lung function[590]. Previously reported correlation coefficients between sputum eosinophils and FeNO range from 0.35 to 0.48 during periods of stability[555,589,591,593]. When symptomatic, a correlation of 0.62 has been reported by Jones and colleagues, who induced loss of control following steroid withdrawal in 60 mild to moderate asthma patients[590]. In patients hospitalised with AAs, Bjerregaard et al.[594] reported a correlation coefficient of 0.57. In the MEX study, McDowell et al. reported a correlation coefficient of 0.56 in exacerbations in a severe cohort on Mepolizumab[455]. The correlation coefficient of 0.59 identified in the present study was similar to those identified in symptomatic and exacerbating asthma patients. The optimal FeNO cutoff for detecting sputum eosinophilia identified in the present study (49 ppb) is just above the normal range of FeNO reported in well-controlled asthma (22-44 ppb)[595], close to the 47 ppb cutoff previously identified as a good indicator of steroid responsiveness[596], and similar to that reported by the ATS clinical practice guideline (50 ppb) as indicative of eosinophilic inflammation and likely steroid responsiveness in symptomatic patients[551]. The MEX study[455] investigated the inflammatory profiles of exacerbations in patients on Mepolizumab and identified that a FeNO cutoff of 50 ppb identified those with sputum eosinophilia (SEC ≥2%) with a positive predictive value of 77%, similar to that observed in the present study (66%). The findings reported in this study have not been reported in a real-life setting of

unselected acute asthma episodes, and many of the studies referenced above included stable patients or were performed in well-controlled research settings. The present study included a random population sample of patients admitted acutely with suspected AAs, and hence, the findings are reflective of "real life" clinical practice.

Raised FeNO levels are associated with worse asthma symptoms[597,598], increased risk of exacerbations[598,599], frequent systemic steroid bursts[600], and FeNO-based management of stable asthma reduces the risk of AAs[601-604]. In addition, Jones et al.[590] demonstrated that periods of loss of control are characterised by increased FeNO levels compared to periods of stability. Further, FeNO levels commonly increase during AAs[584,605,606], and have been shown to decrease following treatment with OCS[605,607]. The reduction in FeNO following the treatment of AAs is typically accompanied by a concomitant improvement in lung function[608]. These observations, along with finding from the present study that FeNO can be a proxy of the underlying inflammatory process during AAs, imply that FeNO (49 ppb, or 50 ppb as per the ATS) has utility as a biomarker in acute asthma, especially as it can be easily and rapidly measured during AAs, and is not readily affected by OCS[607], making it a useful biomarker of T2-high activity even after initiation of OCS. However, this hypothesis needs to be further tested during AAs and periods of recovery following treatment.

It is important to point out that such biomarker-directed management of AAs needs to take into consideration BEC as well; although both (FeNO and BEC) biomarkers reflect T2-high activity, they may reflect activity of different T2-high pathways. Indeed, targeting eosinophilia using anti-IL5 has no effect on FeNO levels[247], and reduction in FeNO following anti-IL4 or IL-13 treatment does not impact BEC levels[239]. In this regard, Pavord et al.[609] suggest stratifying T2-high inflammation on the basis of the prominent driving mechanism (FeNO: magnet vs. BEC: bomb), where FeNO reflects the extent systemic eosinophils are derived to the airway compartment, and high FeNO along with blood eosinophilia synergistically predict exacerbation risk. Nonetheless, these observations are in line with findings from the present study that 27 patients had isolated FeNO increase, five patients had isolated BEC increase, and 19 patients had both biomarkers increase, although this was highly likely to have been influenced by pre-admission and pre-recruitment OCS treatment.

Blood eosinophil count is also a robust, widely used biomarker that correlates well with sputum eosinophils[555]. However, the present study did not find a significant correlation between blood and sputum eosinophils. This is likely explained by the prompt effect steroids have on circulating eosinophils[607,610-612]; 20% of the patients recruited had pre-admission treatment with OCS, and almost all had received a dose of systemic steroids prior to study recruitment. Although the present study found that BEC ≥0.4 $x10^9$/L was a good predictor of SEC ≥3%, the combination of FeNO ≥25 ppb and BEC ≥0.4 $x10^9$/L was a superior predictor than raised BEC alone, in line with previously reported literature highlighting the additive predictive value when both biomarkers are elevated[474,475,613-615].

Heterogeneity of inflammatory phenotypes of asthma attacks

Our study further confirmed the heterogeneity of inflammatory profiles of AAs, a well-recognised but poorly understood phenomenon. 35% of the patients had T2-high inflammation, 13% had T2-low inflammation, 22% had mixed (i.e. T2-high and neutrophilic) inflammation, and 30% had a paucigranulocytic profile. These findings align with existing literature[220,256,445,455,616,617] and challenge the current approach of treatment targeting acute episodes of asthma uniformly.

Perhaps the best demonstration of the extent of the heterogeneity of AAs to date is the findings of the MEX study[618] that demonstrated heterogeneity of AAs even in a *well-controlled* T2-high environment. Whilst some patients (52%) on Mepolizumab had T2-low AAs, others (48%) had non-IL-5 driven T2-high (likely IL-4/IL-13 driven) AAs characterised by increased FeNO levels.

The small airways

Asthma has long been recognised as a disease of the large airways; however, evidence suggests that SAD is important in the pathophysiology of asthma. Although it is not clear how SAD contributes to the pathophysiology and clinical manifestation of AAs, the present study identified at least 62% of those admitted with AAs had evidence of small airway obstruction.

Prior evidence suggests small airways pathology may be important in the pathophysiology of AAs. Thompson et al.[619] assessed acinar and conductive ventilation

heterogeneity using breath nitrogen washout test in hospitalised compared to stable asthma patients and reported an increased conductive ventilation heterogeneity in exacerbating patients compared to stable patients. Similar findings have also been reported in children with asthma[326]. Further, Segal et al. assessed proximal and distal airway reactivity in response to methacholine challenge testing using both spirometry and FOT at baseline and after MCT in asthma and COPD patients[620]. Total and small airway resistance was higher in patients with positive MCT (PC20 ≤16 mg/mL) compared to those without. Of note, the resistance of the large airways was similar to that of those with and without AHR. In those who developed symptoms during MCT, 9 (27.3%) had minimum FEV_1 change and increased total airway resistance was largely secondary to an increase in small airway resistance, suggesting that symptoms worsening following MCT was chiefly driven by small airway reactivity to MCT, implying an important role of the small airways in symptom development in response to a stimulus. Similar findings have also been reported by Mansur et al.[327].

Further highlighting their potential role in the deterioration of lung function during AAs, the small airways have been demonstrated to be involved in the late-asthmatic response following allergen exposure. Metzger and colleagues[621] performed an allergen bronchoprovocation challenge followed by spirometry measurements over a 24-hour period on 17 asthma patients, of whom 12 developed early and late asthmatic responses. Although forced expiratory flow at 50% of forced vital capacity ($FEF_{50\%}$) immediately increased following the allergen challenge, indicating a large airway early response, there was a gradual decrease in $FEF_{50\%}$ over the 6-24 hour period following allergen exposure, suggesting small airway-driven late asthmatic response; the magnitude of the late response, as measured by changes in FEV_1, tended to directly correlate with the reduction of $FEF_{50\%}$. Similar findings have also been reported by Zeidler and colleagues[332].

In light of this, although it is not clear how SAD contributes to the pathophysiology and clinical manifestation of AAs, the balance of evidence suggests a contribution of small airway pathology towards the symptomatology and lung function decline during AAs. Studies during acute asthma and periods of recovery are required to fully elucidate the role of the lung periphery in the pathophysiology of AAs.

Physiological tests of lung function

Our study identified a discrepancy between spirometry and FOT in identifying airflow obstruction. 16 of 83 patients (25.0%) had isolated R5 obstruction, 21 (32.8%) had isolated obstructive FEV_1/FVC, and 27 (42.2%) had obstruction on both measures. This is likely due to the different capabilities of these measures in identifying airflow obstruction at different sites of the lungs, including the lung periphery.

Spirometry is the most widely used and studied method for the assessment of airway physiology; however, many of the parameters obtained from spirometry are insensitive to diseases of the lung periphery[622]. FEV_1 is a measure of the airflow through large and medium-sized airways[623], and SAD can silently progress until a significant drop in lung function occurs[624,625]. $FEF_{25-75\%}$ has long been recognised as a measure of small airway obstruction; however, in addition to its well-recognised variability and limited reproducibility[112,626], it is highly influenced by other spirometric measures. Quanjer et al.[627] investigated the added value of assessing $FEF_{25-75\%}$ in a large retrospective analysis and found a significant correlation between $FEF_{25-75\%}$ and FVC and FEV_1/FVC. As much of the knowledge of $FEF_{25-75\%}$ comes from studies investigating stable asthma, it is unknown whether this parameter is affected during the acute disease process. However, given the correlation between $FEF_{25-75\%}$ and FEV_1 and FVC noted in Quanjer's study, it perhaps sensible to conclude that $FEF_{25-75\%}$ reduction during AAs is secondary to a reduction in FEV_1 and FVC. Thus, it is difficult to comment on how much of the observed discrepancy is owed to $FEF_{25-75\%}$ alone (i.e., SAD) or the inability of spirometry to detect abnormalities in the small airways. However, since FEV_1 is independent of $FEF_{25-75\%}$ (FEV_1 can be healthy despite impaired $FEF_{25-75\%}$), it is reasonable to postulate that the 21 patients with isolated spirometry impairment (without FOT impairment) represent a group of patients mainly with large airways abnormality without small airways abnormality. The logic behind this is that the measure used to define FOT impairment, R5, actually represents the entire airway area (total airway resistance), and thus, abnormalities at the lung periphery would have been detected by FOT as per the present study's methodology. However, the role of these parameters during AAs is not well understood, and further studies are required to allow a more robust understanding of these measures during acute episodes of asthma.

Another possibility for the isolated FEV_1/FVC limitation is the use of a fixed FEV_1/FVC cutoff value as opposed to the lower limit of normal, which is known to overestimate airflow limitation[628]. However, a fixed cutoff was also used to define airflow obstruction using R5, making it less likely that the fixed FEV_1/FVC cutoff is the reason for this discrepancy. In addition, the deep inhalation and forced expiratory manoeuvre required for spirometry are known to induce vagal bronchoconstrictor reflex mediated airflow limitation[629-631], and thus, together with an already impaired lung function, and the use of a fixed cutoff for FEV_1/FVC could have overestimated the number of patients with isolated spirometry obstruction. Although this possibility is unlikely owing to the clinical presentation of those patients at the time of admission, it cannot be excluded.

Sixteen of the patients had isolated FOT obstruction (without obstruction on spirometry). This is likely due to the greater sensitivity of FOT to changes in lung function or the greater involvement of small airways not detected by conventional spirometry as supported by the raised mean R5-20 value, which was 1.7 cmH2O/L/s.

FOT has been demonstrated to detect abnormalities in airway physiology in patients with preserved spirometry[632,633]. Kim and colleagues[634] compared FOT and spirometry parameters in asthma patients with preserved spirometry and controls to evaluate the applicability of FOT measurement in diagnosing asthma in those with preserved lung function. The study found that compared to FEV_1, the area of reactance had better sensitivity, specificity, positive predictive value, and negative predictive value for clinically diagnosed asthma. The authors concluded that FOT might be useful in diagnosing asthma in those with preserved spirometry. Berger et al.[635] investigated the extent of small airway dysfunction-related respiratory symptoms following the world trade centre destruction dust exposure by assessing lung function using spirometry and FOT in 166 dust-exposed patients following MCT. In those who developed symptoms during MCT, 22% had minimum FEV_1 change but had significant R5 increase secondary to increased R5-R20, highlighting the inability of spirometry to detect small airway-related abnormalities. Further, as FOT has been shown to be of utility in detecting early SAD even when FEV_1/FVC is normal[633], together with the fact that a significant amount of lung periphery dysfunction is required before FEV_1/FVC is impacted[636], it is possible that those with isolated FOT impairment had minimum or early SAD detected by FOT, but not spirometry.

The lack of concordance between measures of airflow limitation assessment identified in the present study has been previously reported. Park et al. assessed the ability of FOT compared to spirometry to estimate airflow obstruction in asthma patients and controls[637]. Although the discriminative ability of R5 was comparable to FEV_1 and FEV_1/FVC, authors noted discordance between the two measures, which they attributed to the difference between the two measures, as spirometry parameters are measured during maximal expiration, whereas FOT is measured during tidal breathing; authors suggested a complementary role of FOT in the assessment of airflow obstruction. Moreover, Sheen and colleagues[638] investigated whether bronchodilator response (BDR) assessed using FOT supplements BDR assessed using spirometry in children with suspected asthma and found that in those with asthma, 18% had isolated spirometry BDR, 9% had isolated FOT BDR, and 8% had both FOT and spirometry BDR. The authors concluded that FOT and spirometry cannot be used interchangeably as spirometry is lung volume dependent and reflects airflow and large airway calibre, while FOT is an effortless lung function measure that assesses airway calibre and small airway function and is independent of respiratory muscles, hence each measure provides information on different aspects of lung mechanics.

Study strengths and limitations

Our study is one of the largest studies characterising hospitalised patients with suspected AAs to date; the number of patients recruited in the present study is comparable to, and often exceeds, the number of patients recruited in studies involving asthma exacerbations in a real-life hospital setting (PRISMA by Couillard et al[475], N= 53 participants; the MEX study by McDowell et al., N= 145 participants; Prevalence of eosinophilia in hospitalized patients with asthma exacerbation by Hasegawa et al.[639], N= 80 participants). In addition, data completeness is a major strength of the present study; the vast majority of the patients recruited completed most of the study assessments, including measures of lung function and inflammation. Further, the study recruited patients right after hospital admission, and study assessments were performed during the very unwell state; thus, findings from the present study reflect acute asthma at the peak of symptom deterioration, although to a lesser extent for those seen at A&E prior to hospital admission. Finally, being a real-world, broadly

inclusive study recruiting patients diagnosed following commonly used clinical practice guidelines, data from the present study reflect a diverse population managed following routine clinical practice, and findings should lead to conclusions highly relevant and applicable to day-to-day clinical practice.

Limitations to the present study mainly arose from the uncontrolled settings. Findings on inflammatory profiles are likely influenced by pre-admission GP-prescribed OCS, pre-admission OCS treatment at A&E, and OCS received soon after admission prior to study recruitment. Likewise, a small number of the patients recruited were taking asthma biologic treatments, which are known to suppress T2-high inflammatory markers. Similarly, FeNO levels are influenced by the diameter of the airway calibre; bronchoconstriction typically results in reduced FeNO levels[640], and bronchodilation and the accompanied improved ventilation releases air trapped in the distal airways, which contributes to FeNO production[641], and thus results in higher FeNO concentration compared to levels prior to bronchodilation[642,643]; hence pre-recruitment bronchodilators could have altered FeNO levels. Similarly, it is likely that those treated at A&E prior to hospital admission were administered bronchodilators, and thus, their lung function could have improved by the time of study recruitment; however, a significant improvement would be unexpected as a "true" asthma attack is not readily abated with bronchodilators[156].

Moreover, although the inflammatory marker cutoffs used are commonly adopted in asthma studies, they are not well-validated in acute asthma settings. The study team acknowledges the potential for misclassification in individuals with normal inflammatory markers at the 95% CI, particularly with FeNO, which has multiple interpretive thresholds. Nevertheless, a minimum FeNO of ≥25 ppb is widely considered indicative of T2 inflammation. Another limitation of the present study is that the diagnosis of asthma was not confirmed. Although this information is important, confirmation of asthma diagnoses is not routinely performed during AA admissions, and all admissions recruited were considered acute asthma based on a primary admission diagnosis of asthma exacerbation. Although evidence suggests that not all asthma patients actually have asthma[500], we suspect this will only apply to a small number of patients as misdiagnosis probably occurs in milder, GP-diagnosed patients treated in the community. Further, although few patients refused to participate in the study,

some of those who did appeared very unwell, and hence, their inclusion could have altered the findings; however, the study team believes that a representative sample of patients admitted was recruited, as demonstrated by a minimum FEV1% of 21.3. In addition, patients with COVID-19 or significant co-existing respiratory diseases were excluded to reduce confounding. Although this may have resulted in missing out some admissions mislabelled as asthma attack, the inclusion of such patients would have introduced potential bias due to overlapping pathologies and altered lung physiology. Further, not all patients were able to perform quality lung function manoeuvres; those with poor patient technique or sub-optimal reliability or repeatability manoeuvres were excluded. In addition, this is a single-centre study, and findings might not be applicable to other settings. Finally, as this is an observational descriptive analysis lacking a control arm, the present study is subject to common limitations of such an observational design.

Several methodological considerations must be acknowledged when interpreting the findings of this study. First, the potential for selection bias exists due to the recruitment of patients from the respiratory ward. While this ensured a specialist-confirmed diagnosis, it may limit the representativeness of the sample and exclude patients admitted under other medical units or discharged directly from A&E. Additionally, response bias is possible if certain patients (those with milder or more severe episodes) were more or less likely to consent to participation or complete study assessments. Moreover, the use of several overlapping markers of inflammation (FeNO, blood eosinophil count, and sputum eosinophils), as well as overlapping measures of lung function (spirometry and FOT) raises the potential for collinearity and statistical dependency between variables. For example, elevated FeNO and BEC both reflect T2-high inflammation and are likely to correlate; similarly, FEV_1 and R5 are both influenced by airway calibre. This could artificially amplify associations or potentially obscure independent effects.

Conclusion

Our results demonstrate that almost 1 in 5 patients admitted to a specialist respiratory centre for suspected asthma attacks do not have objective evidence of airflow obstruction. This is likely to be due to the limited accuracy of the clinical assessment and diagnosis of AAs based on non-asthma-specific symptoms and airflow obstruction using PEFR. The use of more robust measures of lung function can improve the assessment and diagnosis of AAs, and the present study confirmed that objective POC measures of airflow obstruction were well tolerated soon after hospital admission in the vast majority of the patients. The present study also highlighted the potential utility of POC measurement of FeNO in guiding the management of AAs, which could inform future biomarker-directed treatment of AAs. The results further confirmed that AAs are biologically heterogenous as in stable asthma, and as such, the current one-size-fits-all management strategy may not be optimal for all patients.

A significant number of the patients admitted for AAs had small airway impairment. The significance of such a finding is unclear as the role of the small airways in the pathophysiology of AAs is not well understood; however, evidence suggests that the small airways have an important role in asthma pathophysiology. Further studies during AAs and periods of recovery will elucidate whether or not SAD is important during episodes of acute symptom worsening. Further, likely due to the different abilities of lung function measures in assessing different parts and mechanics of the airways, we identified discordance between spirometry and FOT in identifying airflow obstruction. This suggests that for accurate evaluation of the severity of airflow obstruction during AAs, both spirometry and FOT assessment may be required.

Chapter 4: APEX3- Comparison between Likely Acute Asthma-Related Admissions (LAARA, n= 64) and Likely Not Acute-Asthma Admissions (LINAA, n= 19)

Introduction

The clinical diagnosis of asthma attacks is largely based on assessment of non-specific symptoms and potentially unreliable measures of airflow limitation. This is further confounded by the vague definition of AAs and the difficulties distinguishing poor asthma control from genuine AAs. As a result, as we have previously demonstrated (Chapters 2 and 3), a significant proportion of patients admitted with acute asthma do not have objective evidence of airflow limitation suggestive of acute asthma[493,494]. We have previously described and compared the characteristics of patients with and without evidence of acute asthma based on retrospectively collected (and therefore limited) data. The aim of this study is to describe and compare the demographic features, clinical characteristics, and admission details of patients with (LAARA) and without (LINAA) prospectively assessed, objectively defined evidence of airflow limitation suggestive of acute asthma. Further comparisons, using the same methodology described below, between Likely Acute Asthma-Related Admissions (LAARA) and Non-Asthma-Related Admissions (NARA) (Appendix G), LAARA and Likely Out of Control Asthma Admissions (LOCA) (Appendix H), patients with high BMI and low BMI (Appendix I), frequent exacerbators and less frequent exacerbators (Appendix J), will be summarised at the end of this chapter and presented in the Appendices.

Aim

To describe and compare the characteristics of patients admitted with suspected severe acute asthma based on the presence or absence of evidence of airflow limitation suggestive of acute asthma.

Primary Objective

To compare the demographics, medical history, and clinical characteristics between those with and without evidence of airflow limitation suggestive of acute asthma.

Secondary Objectives

- To compare demographic features and medical history between those with and without evidence of airflow limitation suggestive of acute asthma.
- To compare the incidence of extra-pulmonary co-morbidities and risk factors for attacks between those with and without evidence of airflow limitation suggestive of acute asthma.
- To compare lung function and symptoms burden between those with and without evidence of airflow limitation suggestive of acute asthma.

Hypothesis

The characteristics of patients with symptoms suggestive of acute asthma but without evidence of airflow limitation match those described in the Audit and previous descriptions of patients with asthma mimics.

Methods

Much of the methodological design of this study follows that described in Chapter 3. The definitions and statistical analyses performed will be briefly described.

Definitions

Evidence of acute asthma

Evidence of acute asthma was based on the presence of objective evidence of airflow limitation assessed using spirometry (FEV_1/FVC <0.7) and FOT (R5 >5 cmH_2O/L/s) (only patients who completed both measures of airflow obstruction (n= 83) are included in this analysis). Admissions with evidence of airflow limitation are referred to as LAARA (Likely Acute Asthma-Related Admissions n= 64), while those with no evidence of airflow limitation are referred to as LINAA (Likely Not Acute-Asthma Admissions, n= 19).

Statistical analysis

Continuous variables were expressed as mean (SD) for normally distributed data and median (IQR) for skewed distribution. Distribution of data was visually assessed. Categorical data were expressed as numbers and percentages. Comparisons between continuous, normally distributed variables was performed using two-samples t-test. For skewed variables, a Wilcoxon signed-rank test was performed if data could not be

transformed. Categorical data were compared using Chi-squared test or Fisher's exact where expected values are below 5. Number of observations presented will vary depending on available data.

Results

Patients' demographics and medical history

The majority of the patients identified were white females, with a mean age of 43.2 years. No significant differences were observed in age, gender, or ethnicity between the groups (Table 33).

Table 33. Demographic characteristics of patients with and without evidence of acute asthma.

Demographics	N= 83 (100%)	LAARA (n= 64, 77.1%)	LINAA (n= 19, 22.9%)	P value
	N (%) unless otherwise stated			
Age (Mean ± SD) (years)	43.2 ± 16.6	43.8 ± 16.7	41.1 ± 16.4	0.54
Gender				
• *Male*	26 (31.3)	19 (29.7)	7 (36.8)	0.55
• *Female*	57 (68.7)	45 (70.3)	12 (63.2)	
Ethnicity				
• *White/Caucasian*	59 (71.1)	44 (68.8)	15 (78.9)	0.92
• *Black/Black British*	8 (9.6)	7 (10.9)	1 (5.3)	
• *Asian/Asian British*	13 (15.7)	10 (15.6)	3 (15.8)	
• *Other*	3 (3.6)	3 (4.7)	0 (0)	

Patients in the LINAA group tended to be more likely to have a previous diagnosis of asthma (100% vs. 85.9%, P= 0.11). No differences were observed in the proportion of patients with atopic diseases or significant comorbidities. Similarly, there was no difference in the proportion of concurrent diagnosed airway diseases, asthma mimics, or allergies between the groups (Table 34). A summary of missing data is presented in Appendix F.

Table 34. Medical history and comorbidities in patients with and without evidence of acute asthma.

Medical history and comorbidities	N= 83 (100%)	LAARA (n= 64, 77.1%)	LINAA (n= 19, 22.9%)	P value
	N (%) unless otherwise stated			
Previously diagnosed asthma	74 (89.2)	55 (85.9)	19 (100)	0.11
Atopic comorbidities	36 (43.4)	26 (40.6)	10 (52.6)	0.35
• *Eczema*	18 (21.7)	12 (18.8)	6 (31.6)	0.34
• *Rhinitis*	28 (33.7)	19 (29.7)	9 (47.4)	0.15
Significant comorbidities	63 (75.9)	47 (73.4)	16 (84.2)	0.54
• *Depression*	38 (45.8)	28 (43.8)	10 (52.6)	0.49
• *Anxiety*	33 (39.8)	24 (37.5)	9 (47.4)	0.44
• *Nasal polyps*	4 (4.8)	2 (3.1)	2 (10.5)	0.22
• *GERD*	22 (26.5)	15 (23.4)	7 (36.8)	0.24
• *Hypertension*	13 (15.7)	11 (17.2)	2 (10.5)	0.72
• *Obesity (BMI ≥30)*	40 (48.2)	28 (43.8)	12 (63.2)	0.13
• *BMI (Mean ± SD)*	30.9 ± 8.4	30.3 ± 8.9	32.8 ± 5.8	0.14
Airway diseases	7 (8.4)	5 (7.8)	2 (10.5)	0.66
• *COPD*	1 (1.2)	1 (1.6)	0 (0)	1
• *Sleep apnoea*	5 (6.0)	3 (4.7)	2 (10.5)	0.32
• *EDAC*	1 (1.2)	1 (1.6)	0 (0)	1
Asthma Mimics	5 (6.0)	3 (4.7)	2 (10.5)	0.32
• *ILO*	3 (3.6)	2 (3.1)	1 (5.3)	0.55
• *Dysfunctional breathing*	1 (1.2)	0 (0)	1 (5.3)	0.22
• *EDAC*	1 (1.2)	1 (1.6)	0 (0)	1
Smoking history				
• *Never*	46 (55.4)	36 (56.3)	10 (52.6)	0.31
• *Current*	16 (19.3)	14 (21.9)	2 (10.5)	
• *Ex smoker*	21 (25.3)	14 (21.9)	7 (36.8)	
Latex/alcohol allergy	5 (6.0)	4 (6.3)	1 (5.3)	1
Medication allergies	30 (36.1)	24 (37.5)	6 (31.6)	0.63

GERD: Gastroesophageal reflux disease. BMI: Body Mass Index. COPD: Chronic obstructive airway disease. EDAC: Excessive dynamic airway collapse. ILO: Inducible laryngeal obstruction.

Asthma-specific history

Asthma-specific history in those with previously diagnosed asthma (n= 74) is shown in Table 35. Asthma age of onset, the number of years with asthma, the presence of a family history of asthma, and allergies were similar between the groups. There was no difference in the number of patients who have asthma action plans and those who know how to respond to a low PEFR level between the groups. The number of patients

who owned pets tended to be slightly increased in the LINAA group compared to the LAARA group (P= 0.08). A summary of missing data is presented in Appendix F.

Table 35. Asthma-specific history in patients with and without evidence of acute asthma.

Previously diagnosed asthma	N= 74	LAARA (n= 55)	LINAA (n= 19)	P value
Asthma-specific history	**N (%) unless stated otherwise**			
Age onset (in years) Median (IQR)	9.5 (0 – 26.5)	10 (4 – 25)	6 (0 – 30)	0.36
Years with asthma (Mean ± SD)	24.7 ± 13.9	24.8 ± 14.2	24.7 ± 13.7	0.99
Family history of asthma	36 (48.7)	29 (52.7)	7 (36.8)	0.39
Perceived allergies	61 (82.4)	45 (81.8)	16 (84.2)	1
• *Animals (Cats, Dogs)*	31 (41.9)	22 (40.0)	9 (47.4)	0.57
• *Inhaled allergens (pollen, house dust mites)*	59 (79.7)	43 (78.2)	16 (84.2)	0.75
• *Grass, Trees*	33 (44.6)	22 (40.0)	11 (57.9)	0.18
• *Other: Mold, Seafood, Nuts*	3 (4.1)	2 (3.6)	1 (5.3)	1
• Aspirin allergy	13 (17.6)	10 (18.2)	3 (15.8)	1
Has asthma action plan	23 (31.1)	16 (29.1)	7 (36.8)	0.53
Has peak flow meter	53 (71.6)	40 (72.7)	13 (68.4)	0.71
Knows how to respond to a low PEFR	39 (52.7)	27 (49.1)	12 (63.2)	0.29
Pet ownership	34 (45.9)	22 (40.0)	12 (63.2)	0.08

PEFR: peak expiratory flow rate.

Asthma medications

Asthma treatment in those with previously diagnosed asthma is shown in Table 36. There was no difference in the proportion of patients on asthma treatment at the time of study recruitment. Similarly, there was no difference in the number of patients on an asthma biologic treatment or low-dose OCS at the time of study recruitment.

Table 36. Asthma treatment in patients with and without evidence of acute asthma.

Previously diagnosed asthma	N= 74	LAARA (n= 55)	LINAA (n= 19)	P value
Asthma treatment	N (%)			
Not on asthma treatment	9 (12.2)	8 (14.6)	1 (5.3)	0.43
SABA only	4 (5.4)	3 (5.5)	1 (5.3)	1
On GINA treatment Step	61 (82.4)	44 (80.0)	17 (89.5)	0.49
• *Step 1*	1 (1.6)	0 (0)	1 (5.8)	0.31
• *Step 2*	4 (6.6)	3 (6.8)	1 (5.8)	
• *Step 3*	10 (16.4)	6 (13.6)	4 (23.5)	
• *Step 4*	27 (44.3)	22 (50.0)	5 (29.4)	
• *Step 5*	19 (31.2)	13 (29.6)	6 (35.3)	
Biologics	3 (4.9)	2 (4.6)	1 (5.9)	1
Low-dose maintenance OCS	2 (3.3)	1 (2.3)	1 (5.9)	0.48
Biologics or Low-dose maintenance OCS	5 (8.2)	3 (6.8)	2 (11.8)	0.6

SABA: Short-Acting Beta-Agonists. GINA: Global initiative for asthma. OCS: Oral corticosteroids.

Asthma attack history

Asthma attack history in those with previously diagnosed asthma is shown in Table 37. The proportion of those with >1 unscheduled visit for asthma, >1 OCS course, and >1 AA in the year prior to study recruitment was similar between the groups (Table 37). The number of unscheduled visits, OCS courses, and AAs in the year prior to study recruitment tended to be higher in the LINAA. The proportion of those who previously had admissions for asthma (ever) was similar between the groups. The number of non-ITU asthma admissions in the last 12 months tended to be higher in the LINAA.

Table 37. Asthma attack history in patients with and without evidence of acute asthma.

Previously diagnosed asthma	N= 74	LAARA (n= 55)	LINAA (n= 19)	P value
Asthma-attack history	N (%) unless stated otherwise			
Number of unscheduled visits (A&E, GP, etc) last 12 months Median (IQR)	2 (0 – 4.8)	2 (0 – 4)	3 (1 – 6)	0.19
>1 unscheduled visits (A&E, GP, etc) last 12 months	43 (58.1)	31 (56.4)	12 (63.2)	0.60
Number of OCS courses last 12 months Median (IQR)	1.5 (0 – 4)	1 (0 – 4)	1 (2 – 6)	0.22
>1 OCS courses last 12 months	37 (50.0)	26 (47.3)	11 (57.9)	0.42
Number of AAs last 12 months Median (IQR)	2 (0.3 – 4.8)	2 (0 – 4)	2 (1 – 6)	0.18
>1 AAs last 12 months	43 (58.1)	31 (56.4)	12 (63.2)	0.60
Previous admissions for asthma	53 (71.6)	41 (74.6)	12 (63.2)	0.34
• *Number of previous admissions (ever)* Median (IQR)	3 (1 – 7)	3 (1 – 7)	4.5 (1.8 – 17.5)	0.35
• *Number of NON-ITU admissions* last 12 months Median (IQR)	1 (0 – 1)	0 (0 – 1)	1 (0.8 – 1. 3)	0.20
• *>1 NON-ITU admissions* last 12 months	12 (16.2)	9 (16.4)	3 (15.8)	1
• *Previous ITU admission (ever)*	12 (22.6)	10 (24.4)	2 (16.7)	0.71
• *Previously Intubated (ever)*	4 (7.6)	3 (7.3)	1 (8.3)	1

OCS: Oral corticosteroids. ITU: Intensive Treatment Unit.

Admission characteristics (visit 1)

The length of time from hospital admission to study recruitment and the length of hospital stay were not different between the groups (Table 38). Discharge diagnoses, secondary diagnoses, pre-admission treatment with OCS, and pre-recruitment nebulisation were also not different between those with and without evidence of acute asthma (Table 38). A summary of missing data is presented in Appendix F.

Table 38. Admission characteristics in patients with and without evidence of acute asthma.

Admission characteristics	N= 83 (100%)	LAARA (n= 64, 77.1%)	LINAA (n= 19, 22.9%)	P value
	N (%) unless stated otherwise			
Time to recruitment (in hours) Median (IQR)	8.4 (2.8 – 14.6)	7.9 (2.6 – 15.1)	8.4 (6.8 – 13.5)	0.89
Length of stay in days Median (IQR)	2 (1 – 4)	2 (1 – 4)	2 (0.5 – 4.5)	0.89
Different Primary discharge diagnosis*	8 (9.6)	7 (10.9)	1 (5.3)	0.67
Secondary Diagnosis**	9 (10.8)	6 (9.4)	3 (15.8)	0.42
Pre-admission OCS	16 (19.3)	13 (20.3)	3 (15.8)	1
Pre-recruitment nebuliser	75 (90.4)	58 (90.6)	17 (89.5)	1
On systemic anti-inflammatory†	20 (24.1)	15 (23.4)	5 (26.3)	0.76

**Lower respiratory tract infections, inducible laryngeal obstruction, community-acquired pneumonia, influenza A infection, viral bronchitis, eosinophilic pneumonia. **Potentially relevant to presenting symptoms: Long covid, viral induced wheeze, type 2 respiratory failure, infective exacerbation of asthma, undiagnosed COPD, an element of upper airway wheeze and anxiety, congestive cardiac failure/obesity hyperventilation syndrome, anxiety/hyperventilation, upper airway noise, possible dysfunctional breathing, multifactorial shortness of breath. †At the time of study recruitment: Low-dose OCS, biologics, or pre-admission treatment with OCS.*

Clinical investigations

Pulmonary traits

Physiological parameters

Lung function measures

As expected, due to the parameters used to classify the groups, lung function measures, including FEV_1%, FEV_1/FVC, and PEFR% were significantly higher in the LINAA compared

to the LAARA. The proportion of patients with impaired measures of lung function was higher in the LAARA (Table 39). 16 (25.0%) of the patients in the LAARA had normal lung function ratio and were considered LAARA based on FOT limitation only.

Table 39. Spirometry parameters in patients with and without evidence of acute asthma.

Spirometry parameters	N= 83 (100%)	LAARA (n= 64, 77.1%)	LINAA (n= 19, 22.9%)	P value
	N (%) unless stated otherwise			
On admission FEV_1% (Mean ± SD)	65.8 ± 22.3	58.9 ± 19.4	89.4 ± 13.5	**<0.001**
On admission FVC% (Mean ± SD)	79.0 ± 19.1	75.0 ± 18.5	92.5 ± 14.6	**<0.001**
On admission FEV_1/FVC (Mean ± SD)	67.6 ± 13.9	64.0 ± 13.7	79.5 ± 4.5	**<0.001**
On admission PEFR% (Mean ± SD)	79.5 ± 23.6	74.9 ± 24.5	94.6 ± 11.1	**<0.001**
FEV_1 <80%	60 (72.3)	56 (87.5)	4 (21.1)	**<0.001**
FVC <80%	44 (53.0)	40 (62.5)	4 (21.1)	**0.001**
FEV_1 >80% and FVC <80%	1 (1.2)	0 (0)	1 (5.3)	0.23
FEV_1/FVC >70%	35 (42.2)	16 (25.0)	19 (100)	
PEFR <75%	33 (39.8)	32 (50.0)	1 (5.3)	**<0.001**

On admission R5 was significantly increased in the LAARA (P <0.001); 21 (32.8%) patients from the LAARA had normal R5 and were considered LAARA based on spirometry limitations only (Table 40).

Table 40. Forced oscillation technique parameters in patients with and without evidence of acute asthma.

FOT parameters	N= 83 (100%)	LAARA (n= 64, 77.1%)	LINAA (n= 19, 22.9%)	P value
	N (%) unless stated otherwise			
On admission R5 (Mean ± SD)	5.6 ± 2.4	6.2 ± 2.3	3.6 ± 0.85	**<0.001**
R5 <5	40 (48.2)	21 (32.8)	19 (100)	

Small airways disease

On admission $FEF_{25-75\%}$ was significantly lower, and R5-R20 was significantly higher, in the LAARA compared to the LINAA (Table 41). The proportion of those with small airway disease was not different between the groups.

Table 41. Small airway involvement in patients with and without evidence of acute asthma.

Small airway disease	N= 83 (100%)	LAARA (n= 64, 77.1%)	LINAA (n= 19, 22.9%)	P value
	N (%) unless stated otherwise			
On admission $FEF_{25-75\%}$ (Mean ± SD)	48.1 ± 29.5	37.7 ± 23.9	83.1 ± 16.8	**<0.001**
On admission R5-R20 (Mean ± SD)	1.7 ± 1.6	2.1 ± 1.6	0.5 ± 0.6	**<0.001**
$FEF_{25-75\%}$ <65%	57 (68.7)	55 (85.9)	2 (10.5)	**<0.001**
R5-R20 >1	49 (59.0)	45 (70.3)	4 (21.1)	**<0.001**
Small airway disease*	8 (9.6)	6 (9.4)	2 (10.5)	1

**Defined as per Lipworth's criteria: Preserved FEV_1 (>80%) and (FEF_{25-75} <60% OR R5-20 >1 $cmH_2O/L/s$).*

Breath sounds

Patients in the LINAA were more likely to have a clear chest compared to those in the LAARA (78.9 % vs. 37.5%, P= 0.001). Patients in the LAARA were likely to have wheezing (Table 42). A summary of missing data is presented in Appendix F.

Table 42. Breath sounds in patients with and without evidence of acute asthma.

Breath sounds	N= 83 (100%)	LAARA (n= 64, 77.1%)	LINAA (n= 19, 22.9%)	P value
	N (%)			
Breath sounds: Clear	39 (46.9)	24 (37.5)	15 (78.9)	**0.001**
Breath sounds: Wheezing	42 (50.6)	38 (59.4)	4 (21.1)	**0.03**
Breath sounds: Other	2 (2.4)	2 (3.1)	0 (0)	1

Inflammatory profile

Median blood eosinophil count, FeNO, and sputum eosinophils percentage were all similar between the groups (Table 43). The proportion of those with BEC ≥0.4 $x10^9$/L, FeNO ≥25 ppb, and SEC% ≥3% were also similar between the groups. The median sputum neutrophils count percentage and the proportion of patients with increased sputum neutrophils was also not different between the groups.

T2-high inflammation

The proportion of patients with raised biomarkers of $T2^{APEX3}$ was not different between the groups (P= 0.35). Similarly, the proportion of those with increased biomarkers of $T2^{GINA}$ was similar between the groups (P= 0.57).

Overall inflammation

Overall, 50 (78.1%) of the patients in the LAARA group had raised inflammatory markers ($T2^{APEX3}$ and/or sputum neutrophils) compared to 10 (52.6%) in the LINAA group (P= 0.03) (Table 43). Using $T2^{GINA}$ and/or sputum neutrophils, there was no difference in the proportion of those with increased inflammatory biomarkers between the groups (P= 0.07). A summary of missing data is presented in Appendix F.

Table 43. Inflammatory profiles in patients with and without evidence of acute asthma.

Inflammatory profile	N= 83 (100%)	LAARA (n= 64, 77.1%)	LINAA (n= 19, 22.9%)	P value
	N (%) unless stated otherwise			
BEC Median (IQR) (n=81)	0.15 (0.05 – 0.39)	0.16 (0.05 – 0.43)	0.14 (0.02 – 0.33)	0.45
FeNO Median (IQR) (n=80)	25 (11.8 – 57.5)	26 (12 – 60)	24 (10.5 – 44.0)	0.70
SEC% Median (IQR) (n=45)	0.25 (0.0 – 3.75)	0.25 (0.0 – 4.38)	0.25 (0.06 – 0.25)	0.44
SNC% Median (IQR) (n=45)	88.8 (59.8 – 97.3)	88.5 (60.9 – 97.3)	94 (56.5 – 97.7)	0.86
SNC ≥65%	32 of 45 (71.1)	25 of 35 (71.4)	7 of 10 (70.0)	1
Raised $T2^{APEX3}$	47 (56.6)	38 (59.4)	9 (47.4)	0.35
• BEC ≥0.4 $x10^9$/L	20 of 81 (24.7)	17 of 63 (26.9)	3 of 18 (16.7)	0.54
• FeNO ≥25 ppb	40 of 80 (50.0)	31 of 61 (50.8)	9 of 19 (47.4)	0.79
• SEC ≥3%	14 of 45 (31.1)	12 of 35 (34.3)	2 of 10 (20.0)	0.46
Raised $T2^{APEX3}$ /Neutrophils	60 (72.3)	50 (78.1)	10 (52.6)	**0.03**
Normal $T2^{APEX3}$/Neutrophils	23 (27.7)	14 (21.9)	9 (47.4)	
Raised $T2^{GINA}$	61 (73.5)	48 (75.0)	13 (68.4)	0.57
• BEC ≥0.15 $x10^9$/L	40 of 81 (49.4)	32 of 63 (50.8)	8 of 18 (44.4)	0.63
• FeNO ≥20 ppb	44 of 80 (55.0)	34 of 61 (55.7)	10 of 19 (52.6)	0.81
• SEC ≥2%	16 of 45 (35.6)	14 of 35 (40.0)	2 of 10 (20.0)	0.29
Raised $T2^{GINA}$ /Neutrophils	70 (84.3)	57 (89.1)	13 (68.4)	0.07
Normal $T2^{GINA}$ /Neutrophils	13 (15.7)	7 (10.9)	6 (31.6)	

BEC: Blood eosinophil count. FeNO: fractional exhaled nitric oxide. SEC: sputum eosinophils count. SNC: sputum neutrophils count.

Cytokine profiling

Levels of T2-high cytokines were similar between the groups except for IL-13 which was significantly higher in the LAARA compared to the LINAA (P= 0.02) (Table 44). The concentration of epithelial derived cytokines, Th-17 cytokines, pro-inflammatory cytokines, and interferons were not different between the groups.

Table 44. Cytokines profiling in patients with and without evidence of acute asthma.

Cytokine profiles	N= 83 (100%)	LAARA (n= 64, 77.1%)	LINAA (n= 19, 22.9%)	P value
T2-high cytokines	Median (IQR)			
• IL-5 Median (IQR) (n= 32)	2.8 (1.6 – 6.0)	3.1 (1.7 – 5.7)	2.3 (1.6 – 6.8)	0.81
• IL-13 Median (IQR) (n= 55)	228.6 (104.6 – 367.6)	244.9 (149.4 – 367.6)	86.3 (44.9 – 426.2)	**0.02**
• IL-4 Median (IQR) (n= 67)	41.8 (32.5 – 59.3)	44.4 (34.1 – 57.5)	35.1 (28.3 – 64.4)	0.31
Epithelial derived cytokines				
• IL-33 Median (IQR) (n= 64)	74.1 (49.2 – 108.1)	75.7 (49.2 – 122.5)	68.1 (54.7 – 99.4)	0.77
• TSLP Median (IQR) (n= 67)	1.5 (1.0 – 2.4)	1.7 (1.1 – 2.2)	1.3 (0.97 – 3.6)	0.98
Th17 cytokines				
• IL-17 Median (IQR) (n= 67)	395.6 (250.9 – 563.3)	381.4 (248.8 – 521.8)	395.6 (272.4 – 647.9)	0.49
Pro-inflammatory cytokines				
• IL-8 Median (IQR) (n= 56)	1490.1 (697.6 – 2656.5)	1459.4 (747.7 – 2628.3)	1615.9 (618.9 – 2625.1)	0.97
• IL-1β Median (IQR) (n= 67)	55.9 (23.5 – 120.3)	46.6 (20.8 –115.9)	69.5 (31.3 – 116.3)	0.49
• IL-6 Median (IQR) (n= 67)	21.3 (8.1 – 39.0)	20.7 (8.5 – 46.2)	24.0 (9.9 – 35.9)	0.87
• TNF-α Median (IQR) (n= 67)	14.2 (7.3 – 30.3)	13.6 (7.5 – 31.6)	17.8 (7.7 – 27.8)	0.80
Interferons				
• IFN-α Median (IQR) (n= 67)	10.4 (6.7 – 21.4)	10.4 (6.7 – 18.2)	11.7 (6.7 – 28.4)	0.57
• IFN-γ Median (IQR) (n= 66)	4.9 (2.9 – 8.6)	4.8 (3.2 – 7.4)	5.5 (2.6 – 11.0)	0.91
• IFN-β Median (IQR) (n= 39)	15.4 (3.9 – 21.6)	12.9 (3.9 – 19.0)	19.4 (3.6 – 33.6)	0.43

Evidence of infections

1 (7.1%) patient in the LINAA group had positive sputum MC&S compared to 4 (11.8%) in the LAARA group. The proportions of those with positive throat swab, increased C-reactive protein, and increased blood neutrophils count were not different between

the groups (Table 45). Overall, the proportion of those with infection (Positive sputum MC&S, positive PCR throat swab, or increased CRP or BNC) was not different between the groups. A summary of missing data is presented in Appendix F.

Table 45. Evidence of infection in patients with and without evidence of acute asthma.

Infection status	N= 83 (100%)	LAARA (n= 64, 77.1%)	LINAA (n= 19, 22.9%)	P value
	N (%)			
Positive sputum MC&S	5 of 48 (10.4)	4 of 34 (11.8)	1 of 14 (7.1)	1
Positive PCR throat swab	9 of 37 (24.3)	9 of 30 (30.0)	0 of 7 (0)	0.15
CRP (>10 mg/L)	29 of 82 (35.4)	25 of 64 (39.1)	4 of 18 (22.2)	0.19
BNC (>8 x10^9/L)	26 of 81 (32.1)	21 of 63 (33.3)	5 of 18 (27.8)	0.59
Overall positive infection status*	46 (55.4)	37 (57.8)	9 (47.4)	0.42

*MC&S: Sputum microscopy culture sensitivities. CRP: C-reactive protein. BNC: Blood neutrophil count. *Positive sputum MC&S, PCR throat swab, or increased CRP or BNC.*

Symptoms score

The overall mean Acute Asthma Quality of Life Questionnaire score was not different between the groups. The burden of symptoms was not significantly different but tended to be slightly increased in the LINAA group (P= 0.15). The emotional domain score was not different between the groups (Table 46).

Table 46. Acute asthma quality of life questionnaire assessed on admission in patients with and without evidence of acute asthma.

AAQLQ score	N= 83 (100%)	LAARA (n= 64, 77.1%)	LINAA (n= 19, 22.9%)	P value
	Mean ± SD			
AAQLQ overall score (n=82)	3.9 ± 1.5	3.9 ± 1.6	4.0 ± 1.0	0.76
• *Symptoms score*	4 ± 1.5	3.9 ± 1.6	4.4 ± 0.9	0.15
• *Emotional score*	3.9 ± 1.7	3.9 ± 1.7	3.6 ± 1.4	0.42

Extra-pulmonary traits

Extra-pulmonary comorbidity questionnaires

Extra-pulmonary comorbidity questionnaires were not different between the groups (Table 47). A summary of missing data is presented in Appendix F.

Table 47. Extrapulmonary comorbidity questionnaires assessed on admissions in patients with and without evidence of acute asthma.

Extra-pulmonary comorbidity questionnaires	N= 83 (100%)	LAARA (n= 64, 77.1%)	LINAA (n= 19, 22.9%)	P value
	N (%) unless stated otherwise			
SNOT-22 score (Mean ± SD) (n=54)	44.2 ± 24.2	44.4 ± 25.0	43.5 ± 21.7	0.91
• Mild (8-20)	9 of 52 (17.3)	8 of 42 (19.1)	1 of 10 (10)	0.9
• Moderate (>20-50)	22 of 52 (42.3)	17 of 42 (40.5)	5 of 10 (50)	
• Severe (>50)	21 of 52 (40.4)	17 of 42 (40.5)	4 of 10 (40)	
• SNOT-22 >8* or Rhinitis diagnosis	80 (96.4)	61 (95.3)	19 (100)	1
GERDQ score (Mean ± SD) (n= 60)	6.2 ± 2.4	6.1 ± 2.2	6.4 ± 3.1	0.76
• GERDQ >8	7 of 60 (11.7)	5 of 48 (10.4)	2 of 12 (16.7)	0.61
• GERDQ >8 or GERD diagnosis	29 (34.9)	20 (31.3)	9 (47.4)	0.19
PVCDI score Median (IQR) (n=78)	3 (2 – 6)	3 (2 – 6)	2 (2 – 5)	0.46
• PVCDI score >3	36 of 78 (46.2)	29 of 60 (48.3)	7 of 18 (38.9)	0.48
• PVCDI score >3 or VCD diagnosis	39 (46.9)	31 (48.4)	8 (42.1)	0.63
Nijmegen score (Mean ± SD) (n=81)	21.5 ± 12.7	22.1 ± 13.3	19.7 ± 10.6	0.43
• Nijmegen Index >23	34 of 81 (41.9)	26 of 62 (41.9)	8 of 19 (42.1)	0.9
• Nijmegen Index >23 or Dysfunctional breathing diagnosis	35 (42.2)	26 (40.6)	9 (47.4)	0.6
HADS anxiety score Median (IQR) (n=50)	4 (2 – 6.8)	4 (2 – 7.3)	3 (2 – 5.5)	0.44
• HADS anxiety score >11	7 of 50 (14.0)	6 of 40 (15.0)	1 of 10 (10.0)	1
• HADS anxiety score >11 or anxiety diagnosis	40 (48.2)	30 (46.9)	10 (52.6)	0.66
HADS depression score Median (IQR) (n=45)	3 (1 – 5)	3 (0 – 5.3)	3 (1 – 5)	0.68
• HADS depression score >11	0 of 45 (0)	0 of 36 (0)	0 of 9 (0)	
• HADS depression score >11 or depression diagnosis	38 (45.8)	28 (43.8)	10 (52.6)	0.49

SNOT-22: Sino-nasal outcome test-22 questionnaire. GERDQ: Gastroesophageal reflux disease (GERD) questionnaire. PVCDI: Pittsburgh Vocal Cord Dysfunction Index. HADS: Hospital Anxiety and Depression Scale.

BMI

The mean BMI was not different between the groups. The proportions of BMI categories were significantly different between the groups. The proportion of obese patients tended to be increased in the LINAA group compared to the LAARA group, but

the proportion of overweight patients was significantly higher in the LINAA (P= 0.004) (Table 48).

Vitamin D

Mean Vitamin D levels were slightly increased in the LINAA group, but this was not significantly different (P= 0.17). The proportion of those with vitamin D insufficiency (20-50 nmol/L) and deficiency (<25 nmol/L) was not different between the groups (Table 48).

Table 48. Body mass index and vitamin D in patients with and without evidence of acute asthma.

Other measures	N= 83 (100%)	LAARA (n= 64, 77.1%)	LINAA (n= 19, 22.9%)	P value
	N (%) unless stated otherwise			
BMI (Mean ± SD)	30.8 ± 8.4	30.3 ± 8.9	32.8 ± 5.8	0.14
BMI ranges				
• *Underweight (<18.5)*	1 (1.2)	1 (1.6)	0 (0)	0.03
• *Healthy range (18.5 – 24.9)*	19 (22.9)	19 (29.7)	0 (0)	
• *Overweight (25 – 29.9)*	23 (27.7)	16 (25.0)	7 (36.8)	
• *Obesity (30 – 39.9)*	28 (33.7)	19 (29.7)	9 (47.4)	
• *Severe obesity (≥40)*	12 (14.5)	9 (14.1)	3 (15.8)	
BMI ≥25 (overweight)	63 (75.9)	44 (68.8)	19 (100)	0.004
BMI ≥30 (obesity)	40 (48.2)	28 (43.8)	12 (63.2)	0.14
Vitamin D (Mean ± SD) (n=81)	50.8 ± 27.1	48.7 ± 28.1	57.6 ± 22.6	0.17
• *Vitamin D insufficiency (25-50 nmol/L)*	27 of 81 (33.3)	23 of 62 (37.1)	4 of 19 (21.1)	0.19
• *Vitamin D deficiency (<25 nmol/L)*	15 of 81 (18.5)	13 of 62 (20.9)	2 of 19 (10.5)	0.50

Risk and behavioural traits

Medication adherence report scale

The medication adherence report scale score was not different between the groups (Table 49). A summary of missing data is presented in Appendix F.

Medication Possession Ratio

The median medication possession ratio tended to be slightly increased in the LINAA group compared to the LAARA (100% vs. 50%, P= 0.12) (Table 49).

Inhaler technique

Inhaler technique score was not different between the groups; however, none of those in the LINAA group had poor technique compared to 14.3% in the LAARA (Table 49).

Table 49. Assessment of adherence in patients with and without evidence of acute asthma.

Previously diagnosed asthma	N= 74	LAARA (n= 55)	LINAA (n= 19)	P value
Adherence	N (%) unless stated otherwise			
MARS (n= 72) Median (IQR)	23 (20 – 25)	23 (20 – 25)	23 (22.5 – 24.5)	0.46
MPR (n= 68) Median (IQR)	58 (8.5 – 100)	50 (8.3 – 100)	100 (33 – 100)	0.12
MPR <75%	36 of 68 (52.9)	29 of 51 (56.9)	7 of 17 (41.2)	0.26
On GINA treatment Step	61 (82.4)	44 (80.0)	17 (89.5)	0.49
Inhaler technique (n= 57)				
• *Poor*	6 (10.5)	6 (14.3)	0 (0)	0.16
• *Good*	23 (40.4)	18 (42.9)	5 (33.3)	
• *Excellent*	28 (49.1)	18 (42.9)	10 (66.7)	
• *Total*	57	42	15	

MARS: Medication adherence report scale questionnaire. MPR: Medication Possession Ratio.

Summary of subgroup analyses

Summary of Appendix G: APEX3- Comparison between Likely Acute Asthma-Related Admissions (LAARA, n= 64) and Non-Asthma-Related Admissions (NARA, n= 9)

Demographics and medical history

The NARA group were likely to be younger patients (35.7 ± 8.3 years vs. 43.8 ± 16.7 years, P= 0.03) with slightly increased comorbidities (P= 0.10) and asthma mimics (P= 0.11); concurrent diagnosed airway diseases were similar between the groups (P= 0.56). The proportion of patients with previous asthma diagnosis was not different between the groups although all those in the NARA had previously diagnosed asthma (100% vs. 85.9%, P= 0.59). All those from the NARA were on asthma medications compared to 80% in the LAARA group (P= 0.34); in patients on asthma treatment, a significantly increased number of those in the NARA were on step 5 GINA treatment step (55.6% vs. 29.6%, P= 0.04). The median number of unscheduled visits (A&E, GP, etc), OCS courses, and AAs in the 12 months preceding study recruitment were significantly higher in the NARA compared to the LAARA (6 vs. 2, P= 0.02, 5 vs. 1, P= 0.03, 5 vs. 2, P= 0.04, respectively). The proportion of patients with >1 unscheduled visit, >1 OCS course, and >1 AA in the 12 months prior to study recruitment tended to be higher in NARA compared to LAARA (P= 0.07, P= 0.14, P= 0.07, respectively). The median number of non-ITU admissions for asthma in the year prior to study recruitment was significantly higher in the NARA (1.5 vs. 0, P= 0.02).

Admission characteristics (visit 1)

Admission characteristics, including time from hospital admission to study recruitment, length of hospital stay, different primary diagnoses, and secondary diagnoses relevant to presenting symptoms were not different between the groups.

Lung function measures and breath sounds

As expected, as sub-groups were classified based on the presence of evidence of airflow obstruction, mean FEV_1%, FEV_1/FVC were significantly increased, and mean R5 was significantly decreased, in the NARA compared to the LAARA (92.6 vs. 58.9, P= <0.001, 80.9 vs. 64.0, P= <0.001, 3.6 vs. 6.2, P= <0.001, respectively). Similarly, the proportion

of those with normal breath sounds was significantly increased in the NARA compared to the LAARA (77.8% vs. 37.5%, P= 0.03).

Inflammatory profile

Again, as expected, as inflammatory measures were used to classify subgroups, median BEC and FeNO values were higher in the LAARA compared to the NARA (0.16 $x10^9$/L vs. 0.03 $x10^9$/L, P= 0.05, 26 ppb vs. 10 ppb, P= 0.01, respectively). The proportion of those with raised $T2^{APEX3}$ markers was higher in the LAARA compared to the NARA (59.4% vs. 0%, P= <0.001). Cytokine levels were similar between the groups.

Evidence of infections

The proportion of those with infection (Positive sputum MC&S, PCR throat swab, or increased CRP or BNC) was slightly increased in the LAARA group, although this was not significantly different (57.8% vs. 33.3%, P= 0.28).

Symptoms score

The overall mean AAQLQ score was not different between the groups. AAQLQ symptoms and emotional scores were also similar between the groups.

Extra-pulmonary traits

Extrapulmonary comorbidity questionnaire scores were similar between the groups; however, median BMI and the proportion of those with BMI ≥25 (overweight) tended to be slightly increased in the NARA (P= 0.16, P= 0.06, respectively). Similarly, medication possession ratio tended to higher in the NARA compared to the LAARA (100 vs. 50, P= 0.05).

Summary of Appendix H: APEX3- Comparison between Likely Acute Asthma-Related Admissions (LAARA, n= 64) and Likely Out of Control Asthma (LOCA, n= 10)

Demographics and medical history

Demographic characteristics were similar between the groups. LOCA were more likely to have atopic comorbidities (80% vs. 40.6%, P= 0.04), including eczema (50% vs. 18.8%, P= 0.04) and rhinitis (70% vs. 29.7%, P= 0.03). Significant comorbidities, airway diseases,

and asthma mimics were all similar between the groups. Asthma-specific history, asthma treatment, and asthma attack history were also similar between the groups.

Admission characteristics (visit 1)

Time from hospital admission to study recruitment, length of hospital stay, different primary diagnoses, and secondary diagnoses relevant to presenting symptoms were all similar between the groups.

Lung function measures and breath sounds

Again, as sub-groups were categorised based on airflow limitation, mean FEV_1%, FEV_1/FVC were significantly increased, and mean R5 was significantly decreased, in the LOCA compared to the LAARA (86.5 vs. 58.9, P= <0.001, 78.2 vs. 64.0, P= <0.001, 3.5 vs. 6.2, P= <0.001, respectively). The proportion of those with normal breath sounds was significantly increased in the LOCA compared to the LAARA (80% vs. 37.5%, P= 0.02).

Inflammatory profile

Again, as expected, as subgroups were categorised based on inflammatory profiles, median FeNO, and the proportion of those with increased markers of $T2^{APEX3}$ inflammation tended to be higher in the LOCA compared to the LAARA (44 ppb vs. 26 ppb, P= 0.06, 90% vs. 59.4%, P= 0.08, respectively). Median BEC and the proportion of those with increased BEC levels (≥0.4 $x10^9$/L) were similar between the groups. Cytokine levels were largely similar between the groups apart from median IL-13 which was significantly increased in the LAARA compared to the LOCA (244.9 vs. 68.4, P= 0.01).

Evidence of infections

The proportion of those with infection (Positive sputum MC&S, PCR throat swab, or increased CRP or BNC) was similar between the groups.

Symptoms score

There was no difference in AAQLQ scores between the groups.

Extra-pulmonary traits

There was no difference in extrapulmonary comorbidity questionnaires between the groups. The proportion of overweight and obese patients tended to be slightly higher

in the LOCA group (P= 0.05, P= 0.12, respectively). Vitamin D levels and the proportion of those with vitamin D insufficiency was similar in the LOCA compared to the LAARA.

Summary of Appendix I: APEX3- Lung function and inflammation in patients with high body mass index (≥30, HBMI, n= 40) vs. low body mass index (<30, LBMI, n= 43)

Lung function measures

Both mean FEV_1% and FEV_1/FVC were significantly lower in patients with LBMI compared to those with HBMI (60.1 vs. 71.9, P= 0.01, 62.3 vs. 73.2, P <0.001, respectively). Mean R5 value was not different between the groups. Patients with LBMI tended to be likely to have evidence of airflow obstruction as determined by FEV_1/FVC and/or R5 (70% vs. 83.7%, P= 0.13).

Inflammatory profile

The median FeNO value and the proportion of those with FeNO ≥25 ppb were both higher in the LBMI compared to the HBMI group (39 ppb vs. 19.5 ppb, P= 0.02, 61.9% vs. 36.8%, P= 0.02, respectively). The number of patients with raised $T2^{APEX3}$ markers tended to be higher in the LBMI group (65.1% vs. 47.5%, P= 0.11).

Summary of Appendix J: APEX3- Lung function, inflammation, asthma treatment, and medication prescription rate in frequent exacerbators (>1 OCS in the last 12 months, FE, n= 37) vs. less frequent exacerbators (≤1 OCS in the last 12 months, LFE, n= 46)

Lung function measures

Mean FEV_1%, FVC%, and lung function ratio were similar between the groups. The number of patients with impaired FEV_1/FVC tended to be greater in the LFE compared to the FE (65.2% vs. 48.7%, P= 0.12). Mean PEFR% was lower, and the proportion of those with PEFR <75% was higher, in the LFE compared to the FE (73.7 vs. 86.7, P= 0.01, 50% vs. 20.7%, P= 0.002, respectively).

Inflammatory profile

Both median BEC and FeNO were higher in the LFE compared to the FE (0.30 $x10^9$/L vs. 0.06 $x10^9$/L, P= <0.001, 39 ppb vs. 20 ppb, P= 0.06, respectively). The proportion of patients with raised $T2^{APEX3}$ markers was higher in the LFE compared to the FE (73.9%

vs. 35.1%, P= <0.001). The proportion of those who had OCS treatment prior to hospital admission was higher in the FE compared to the LFE (32.4% vs. 8.7%, P= 0.006).

Asthma treatment and medication prescription rate

Frequent exacerbators were more likely to have previously diagnosed asthma (100% vs. 83.4%, P= 0.004) and to be on asthma medications at the time of study recruitment (94.6% vs. 70.3%, P= 0.005). The proportion of patients on GINA step 5 treatment step and biological treatment or low-dose maintenance OCS were significantly higher in the FE compared to the LFE (51.4% vs. 3.9%, P= <0.001, 13.5% vs. 0%, P= 0.05, respectively). Median medication prescription rate was higher, and the proportion of those with MPR <75% was lower in the FE compared to the LFE (100 vs. 16, P= <0.001, 29.4% vs. 76.4%, P= <0.001, respectively).

Discussion

Few significant differences were identified between LAARA and LINAA groups, but some differences between LAARA and NARA groups were identified supportive of the findings of the Audit (Chapter 2). Those with no apparent acute deterioration of asthma are more likely younger patients with a higher incidence of comorbidities and asthma mimics and increased asthma-related healthcare utilisation. In addition, previously diagnosed asthma may contribute to the rate of AA misdiagnosis as all patients with no evidence of acute asthma had previously diagnosed asthma; as such, worsening of asthma-like symptoms is likely attributed to asthma. The present study also found that the level of nasal IL-13 was higher in the admissions with evidence of acute asthma deterioration, raising the potential for IL-13 to be used as a marker of T2-high activity during AAs, but this requires further validation.

Demographic features, medical history and comorbidities of patients with no evidence of acute asthma

Age

Although the main analysis comparing LAARA to LINAA did not find difference in age between the groups, the comparison between LAARA to NARA (Non-Asthma-Related Admissions) (Appendix G) shows that those in the NARA group were significantly younger, consistent with findings of the multiple- and frequent attenders in the Audit, and in line with previous evidence reporting that patients with misdiagnosed AAs[433,494] and those with asthma mimics, including ILO[404,433] and BPD[412], are typically younger females, although the present study did not find a difference in gender between the groups.

Previously diagnosed asthma

Our study identified that all patients without evidence of airflow limitation had previous diagnoses of asthma. The significance of this finding is not clear, but this could imply an association between previously diagnosed asthma and asthma attack diagnoses at subsequent worsening of respiratory symptoms.

An incorrect diagnostic label of asthma may understandably affect the interpretation of later acute healthcare episodes of the incorrectly diagnosed patient. In addition to

the unnecessary treatment with SCS and inhaled steroids, both of which have been associated with adverse side effects[206,209,644,645], a prior diagnosis of asthma may lead healthcare providers to reach the wrong conclusions regarding the cause of acute symptoms early in the patient assessment[526]. Alternative diagnoses and asthma mimics may, thus, be overlooked and mistaken for AAs, though this is difficult to prove as asthma and asthma mimics are known to co-exist[404,487]. Aaron et al.[500] investigated the diagnosis of asthma in 613 Canadian adults and ruled out asthma in 30%, of whom 6% had previously unrecognised alternative diagnoses known to mimic asthma, including ILO, subglottic stenosis, anxiety, hyperventilation, and tracheobronchomalacia. This essentially means that symptoms worsening in such patients could have been attributed to diseases other than asthma. The best example of this comes from the study of Newman and colleagues[433], who described the characteristics of a group of patients with ILO mistaken as asthma. These patients had extensive healthcare utilisation, including A&E visits, hospital admissions, and even intensive care and intubation for presumed asthma for over an average of 4.8 years.

Comorbidities and asthma mimics

Our study demonstrated that mean body mass index and the proportion of obese (BMI ≥30) patients tended to be increased in those with no evidence of airflow limitation. This aligns with the findings of Centurion et al.[493], who demonstrated that in patients hospitalised with asthma and COPD, those with a BMI of ≥30 were almost four times more likely to have no evidence of airflow limitation. Obesity and asthma appear to be linked[646-648], and obese individuals are more likely to receive a physician diagnosis of asthma compared to non-obese[649]. In a cross-sectional epidemiological study, Schachter et al.[650] found that obesity was a risk factor for an asthma diagnosis, wheezing, and asthma medication use, but not objective measures suggestive of asthma, including airway hyperresponsiveness and airflow obstruction. Similarly, Sin et al.[651] investigated the association between obesity and airflow obstruction (defined as FEV_1/FVC <80%) in more than 16000 patients and found that those with the highest BMI had an increased risk of asthma diagnosis, asthma medication use, and dyspnoea on exertion, but not airflow obstruction. Supporting and extending these findings to the setting of acute asthma, the analysis of obese compared to non-obese patients

(Appendix I) shows that those with a BMI ≥30 had significantly higher mean FEV_1% and FEV_1/FVC and a significantly smaller number of patients with reduced FEV_1/FVC compared to those with a BMI of <30.

Obese patients report a higher burden of asthma-like symptoms compared to non-obese individuals[650], likely due to the increased weight and the accompanying excess elastic load on the chest wall, reduced chest wall compliance, reduced lung volumes, and increased work of breathing[652-654]. Findings from the present study support the idea that acute onset symptoms suggestive of an asthma attack are more likely to be misdiagnosed as asthma attacks in obese individuals; however, further research is needed to test this hypothesis.

Our study also identified that the incidence of asthma mimics, particularly BPD, was slightly increased in the NARA group compared to the LAARA (Appendix G). This supports the findings from the Audit that multiple- and frequent attenders were more likely to have ILO and BPD compared to single-time attendees and also aligns with the study of Centurion et al.[493] demonstrating that those with no evidence of airflow obstruction had evidence of spirometry abnormalities suggestive of ILO. In addition, a recent study by Couillard et al.[475] investigated the response to OCS based on inflammatory markers in patients with GINA-defined AAs and reported that the group of patients with no evidence of T2-high activity (BEC <0.15 $x10^9$/L and FeNO <25 ppb) had preserved pre-bronchodilator lung function and greater symptoms suggestive of dysfunctional breathing pattern assessed using the Nijmegen questionnaire, although the present study did not find differences in Nijmegen questionnaire scores between the groups.

Healthcare utilisation

Those with Non-Asthma-Related Admissions (NARA) (Appendix G) were more likely to be severe asthma patients (on GINA treatment step 5) and have significantly increased asthma-related healthcare utilisation, including GP and A&E visits, OCS bursts, and even hospitalisation, compared to those with likely acute asthma-related admissions (LAARA). This was also consistent with the comparison between multiple- and frequent attenders vs single-time attendees from the Audit. In the sub-analysis of frequent compared to less frequent exacerbators (Appendix J), the present study found that

those with >1 OCS in the last 12 months tended to be less likely to have FEV_1/FVC impairment compared to those with ≤1 OCS. This aligns with findings from the Audit that those with multiple AA admissions were less likely to have evidence of acute asthma. Of note, although FEV_1% was slightly higher in the frequent compared to less frequent exacerbators, mean PEFR% was significantly higher, and the number of those with PEFR limitation was significantly lower in the frequent exacerbators group compared to the less frequent exacerbators, in line with the previous discussion (Chapter 3) that PEFR% may overestimate FEV_1% in those with less severe obstruction[576].

As previously discussed (Chapter 2), studies of patients with common asthma mimics frequently describe a group of asthma patients on a high treatment step and frequent healthcare utilisation for presumed asthma[415,433]. Thus, although it is plausible that those with severe disease require more frequent medical attention, whether this frequent healthcare utilisation is asthma-related is a key question. Given that patients in the NARA group had no evidence of airflow limitation and inflammatory markers within normal levels, it is likely that symptoms in those patients are due to pathologies other than asthma. However, further studies during periods of symptoms worsening and recovery are required to confirm such a conclusion.

The potential utility of cytokine profiling

Our study identified that the level of IL-13 was significantly increased in the LAARA compared to the LINAA, which is biologically plausible as IL-13 is a potent T2-high mediator[655] that plays an important role in driving airway eosinophilia[656], and is known to promote airway smooth muscle hyperresponsiveness and contraction[657]. Tsilogianni et al. demonstrated that the level of sputum IL-13 was superior to both FeNO and sputum eosinophils in identifying patients with poorly controlled asthma[658]. IL-13 levels have also been shown to inversely correlate with asthma control assessed using the asthma control test (ACQ)[659]. Further, IL-13 has frequently been reported to be increased during AAs[660-663].

Neutralizing IL-13 activity improves lung function and decreases the risk of exacerbations in those with T2-high inflammation[664], and has been demonstrated to reduce the intensity of early, as well as late asthmatic responses following allergen

challenge[665]. As FeNO, which we have previously demonstrated to be a potentially useful biomarker of T2-high activity during AAs (Chapter 3), is induced and upregulated via IL-13[585], it is reasonable to speculate that IL-13 could serve as a proxy of T2-high activity during AAs, potentially as a biomarker for IL-13 inhibition with Dupilumab, in a similar fashion to the recently published study by Bafadhel et el.[430] targeting eosinophilic exacerbation of airway diseases using Benralizumab. However, further studies are required to test this hypothesis.

Study strengths and limitations

One of the main strengths of the present study is the large number of patients recruited, which often exceeds the number of patients in similar studies. In addition, the inclusion of both spirometry and FOT allowed a comprehensive assessment of the lung function. As such, the study team believes that patients with no evidence of airflow obstruction represent a genuine subset of patients with likely no acute asthma deterioration at the time of hospital admission.

Study limitations include the exclusion of those unable to perform both measures of airflow obstruction, spirometry and FOT. It could be reasonably assumed that more unwell patients would be less able to perform both measures and could have been more likely to be excluded. Although this may be true, it was essential to incorporate such a methodology as spirometry and FOT are known to reflect different aspects of lung mechanics, and impairment detected by spirometry might not be detected by FOT and vice versa (discussed in Chapter 3). However, the study team believes that a representative sample of patients, including some with severe episodes, were recruited.

Further, the possibility of chance findings must also be considered, particularly given the relatively modest sample size in the LINAA, LOCA, and NARA subgroups. In addition, a large number of statistical tests were performed, and thus, findings from the present study are subject to both type 1 and type 2 errors. Although statistical significance thresholds were applied, no formal correction was used for multiple testing, and therefore some associations, particularly those with p-values near 0.05, should be interpreted with caution and treated as hypothesis-generating. As such, only findings thought to be biologically or clinically plausible were highlighted and discussed.

Another important limitation of this study is the absence of pre-admission (baseline) lung function measurements, which limits the ability to determine whether values observed during admission (FEV_1, FEV_1/FVC, R5) reflect true acute deterioration, or instead represent the patient's usual or stable physiological state. This is particularly relevant for patients with fixed airflow limitation, where abnormal spirometry or oscillometry results at admission may not indicate an acute exacerbation but rather underlying disease severity or persistent airflow limitation. Nevertheless, this is partially mitigated by data obtained at visit 2, which was conducted approximately 14 days post-discharge, and is expected to reflect a more stable, baseline lung function status.

Conclusion

Consistent with findings from the Audit, the present study identified a group of young patients with increased comorbidities, asthma mimics, and high healthcare utilisation for asthma who are frequently admitted for asthma-like symptoms and labelled as asthma attacks but lack objective evidence of asthma deterioration. All the patients with no evidence of AAs had previously diagnosed asthma, suggesting a previous asthma diagnosis may influence the physician’s decision regarding the cause of symptoms worsening. The present study identified potential utility of IL-13 as a T2-high biomarker during AAs, as it was higher in the admissions with evidence of acute asthma than those without. However, further studies are required to validate this hypothesis.

Chapter 5: APEX3- Analysis of follow-up visits

Introduction

Despite the well-documented heterogeneity of the inflammatory profiles underlying asthma attacks, these events are uniformly treated using OCS as the mainstay treatment option despite their potentially variable efficacy[512-514] and limited data describing the response to acute asthma treatment following hospitalisation and treatment with OCS. The clinical characteristics of patients admitted for acute asthma were described in Chapters 3 and 4. The aim of this chapter is to describe the clinical and biological response following hospital admission and treatment with OCS for acute asthma.

Aim

To describe the clinical characteristics and biological response during recovery (visit 2) following hospital admission and treatment with OCS for acute asthma (visit 1).

Primary Objective

To describe changes in lung function, inflammatory profile, infection status, and symptoms burden during recovery (visit 2) following hospitalisation for suspected asthma attacks (visit 1).

Secondary Objectives

- Compare changes in lung function, inflammatory profile, infection status, and symptom burden during recovery (visit 2) following hospitalisation for suspected asthma attacks based on the presence or absence of objective evidence of acute asthma at hospital admission (visit 1).
- Compare changes in lung function, inflammatory profile, infection status, and symptom burden during recovery (visit 2) following hospitalisation for suspected asthma attacks based on the inflammatory profile (T2-high vs. T2-low) at hospital admission (visit 1).
- To explore the utility of inflammatory profiling of AAs (at visit 1) in predicting response (at visit 2) to treatment with OCS.

Hypotheses

1. A significant proportion of patients admitted to hospital with suspected asthma attacks do not have evidence of acute airflow limitation.
2. The recovery following treatment with OCS demonstrates significant variation in terms of lung function and symptom recovery based on the underlying inflammatory profile.
3. Point-of-care inflammatory profiling of acute asthma may be of utility in predicting recovery following treatment with OCS.

Methods

Much of the methodological design of this study follows that described in Chapter 3. A summary of study settings, definitions, and statistical analyses performed will be described.

Summary of study setting

Following study recruitment at hospital admission for acute asthma (visit 1), a follow-up visit was arranged (visit 2), which took place approximately two weeks after hospital discharge, at which lung function tests, blood tests, and questionnaires were performed. A further telephone follow-up visit (visit 3) was scheduled around four weeks after hospital discharge, at which the degree of symptomatic recovery was assessed.

Definitions

Evidence of acute asthma

As in previous chapters (Chapters 3 and 4), evidence suggestive of acute asthma was based on the presence of objective evidence of airflow limitation assessed using spirometry (FEV_1/FVC <0.7) and/or FOT (R5 >5 cmH_2O/L/s).

FEV_1% and FeNO minimal clinically important difference

A minimal clinically important difference (MCID) in FEV_1% was based on the ATS/ERS[666] criteria for a clinically meaningful change for short-term trials, i.e. weeks in duration (FEV_1% ≥20%). Similarly, a change of 20% in FeNO was used as indicative of a MCID at V2 compared to V1 as per the ATS recommendation[551].

Classification of inflammatory markers

As in previous chapters (Chapters 3 and 4), $T2^{APEX3}$ criteria defining T2-high inflammation in the present study is based on any of the following: FeNO level ≥25 ppb, blood eosinophil count of ≥0.4 $x10^9$/L, and sputum eosinophil count of ≥3%.

Statistical analysis

Continuous variables were expressed as mean (SD) for normally distributed data and median (IQR) for skewed distribution. Distribution of data was visually assessed. Categorical data were expressed as numbers and percentages. Between visit changes were compared using a paired sample t Test for continuous, normally distributed variables and paired samples Wilcoxon test if data could not be transformed. McNemar test was used to compare paired categorical data. The continuity-corrected version of the McNemar test was used where the number of discordant pairs is less than 25. Where the number of paired observations was too small to perform a McNemar test, "NA" (not applicable) was used to indicate that the test could not be conducted. Number of observations presented will vary depending on available data.

Between visit changes in the Likely Acute Asthma-Related Admissions (LAARA) compared to the Likely Not Acute-Asthma Admissions (LINAA)

Between visit changes in lung function, inflammation, and symptom scores in the LAARA and LINAA groups were compared using a two-samples t-test for normally distributed variables and Wilcoxon signed-rank test for skewed distribution. A Chi-squared test, or Fisher's exact where expected values are below 5, was used to compare categorical variables.

Predicting a minimum clinically important improvement in FEV_1% (FEV_1% ≥20%)

To determine factors predicting clinically important difference in FEV_1% at V2 compared to V1, a stepwise multivariate logistic model was carried out using R. Factors included are BEC ≥0.15 $x10^9$/L (at V1), BEC ≥0.4 $x10^9$/L (at V1) (note: only one BEC cutoff was included in the multivariate model; if both were significantly associated with the outcome in univariate analysis, the more strongly associated cutoff was selected), FeNO ≥25 ppb (at V1), FeNO >50 ppb (at V1) (note: only one FeNO cutoff was included in the

multivariate model; if both were significantly associated with the outcome in univariate analysis, the more strongly associated cutoff was selected), SEC ≥3% (at V1), PEFR <75% (at V1), wheezing (at V1), and AAQLQ symptom score (at V1). Factors significantly associated (<0.05) with the outcome in the univariate logistic model were included in the multivariate model. A ROC analysis was performed to determine the optimal FeNO cutoff in identifying FEV_1% MCID.

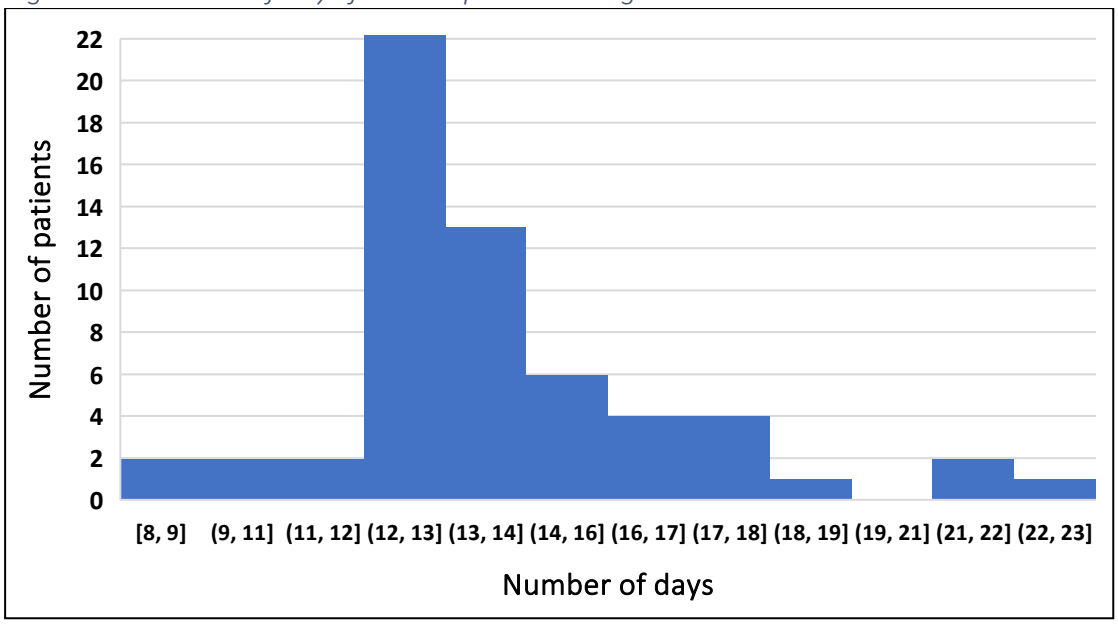
Between visit changes in T2-high compared to T2-low admissions

Between visit changes in lung function, inflammation, and symptom scores in the T2-high compared to T2-low AA admissions (based on $T2^{APEX3}$ criteria: FeNO level ≥25 ppb, blood eosinophil count of ≥0.4 $x10^9$/L, or sputum eosinophil count of ≥3%) were compared using two-samples t-test for normally distributed variables and Wilcoxon signed-rank test for skewed distribution. The Chi-squared test, or Fisher's exact where expected values are below 5, was used to compare categorical variables.

Results

A total of 60 patients completed the follow-up visit (V2), either attended in-person (57, 95.0%) or as a phone call follow-up (3, 5.0%). 37 of 97 (38.1%) patients missed visit 2, and 50 of 97 (51.5%) missed visit 3 (V3). The mean number of days between hospital discharge and V2 is 13.9 days (SD= 2.7 days) (Figure 28). Demographics and clinical characteristics at V2 and between visit changes in all patients who completed V2 will be summarised, and the full analysis will be presented in Appendix K.

Figure 28. Number of days from hospital discharge to V2.



Summary of Appendix K: APEX3- Paired visits in all patients who completed V2 (n= 60)

Demographics of patients at V2

The majority of the patients were white and female with a mean age of 45.7 years. Demographic characteristics of patients at V1 were largely similar to those of patients at V2 (Table 50). A summary of missing data is presented in Appendix L.

Table 50. Demographic characteristics of patients at V2.

Demographics	V1: N= 97	V2: N= 60
	N (%) unless otherwise stated	
Age (Mean ± SD) (years)	43.9 ± 17.0	45.7 ± 16.7
Gender		
• *Male*	32 (33.0)	18 (30.0)
• *Female*	65 (67.0)	42 (70.0)
Ethnicity		
• *White/Caucasian*	69 (71.1)	44 (73.3)
• *Black/Black British*	10 (10.3)	6 (10.0)
• *Asian/Asian British*	13 (13.4)	6 (10.0)
• *Other*	5 (5.2)	4 (6.7)

Clinical investigations at follow-up visits (V2)

Lung function parameters

Of those who completed V2 (n= 60), 57 (95%) attended in person, of whom 47 (82.5%) were able to perform spirometry at both visits. In patients with paired spirometry measurements (n= 47), FEV_1% and FEV_1/FVC were significantly higher at V2 compared to V1 (P <0.001, <0.001, respectively). Of those who completed V2 (n= 60), 57 (95%) attended in person, of whom 56 (98.2%) had paired FOT measurements. The mean R5 value was significantly greater at V2 compared to V1 (P= <0.001).

Of those who completed both measures of airflow obstruction at both visits (n= 47), 21 (44.7%) had normal spirometry and FOT at V2. 26 (55.3%) had evidence of airflow obstruction at V2 on either or both measures compared to 37 (78.7%) at V1 (P= 0.04). Of those with airflow limitation at V2 (n= 26), 9 (19.2%) had normal spirometry and obstructive FOT, 10 (21.3%) had normal FOT and obstructive spirometry, and 7 (14.9%) had evidence of airflow limitation on both measures. $FEF_{25-75\%}$ was significantly higher, and R5-20 was significantly lower at V2 compared to V1 (P <0.001, 0.001, respectively). A summary of missing data is presented in Appendix L.

Inflammatory profile

Paired blood differential count data are available for 55 patients. There was no difference in the median BEC value between visits (0.16 $x10^9$/L vs. 0.20 $x10^9$/L, P= 0.62). There was a significant shift towards a normal BEC at V2 compared to V1 (P <0.001). 55 patients had successfully completed FeNO at both visits. FeNO was significantly lower

at V2 compared to V1 (28 ppb vs. 37 ppb, P= 0.03). The proportion of patients with increased FeNO was not different between visits (P= 0.51). 10 paired sputum samples are available. The median sputum eosinophil percentage was significantly lower at V2 compared to V1 (0.8% vs. 11.8%, P= 0.04). The mean sputum neutrophil count was lower at V1 compared to V2 (62.2% vs. 86.7%, P= 0.02). 32 (56.1%) of the patients had evidence of raised $T2^{APEX3}$ inflammatory markers at V2 compared to 34 (59.7%) at V1 (P= 0.24). 6 of 23 (26.1%) patients with normal markers of $T2^{APEX3}$ inflammation at V1 had increased markers of $T2^{APEX3}$ inflammation at V2. A summary of missing data is presented in Appendix L.

T2-high cytokines, epithelial-derived cytokines, Th-17 cytokines, and interferons were all similar at V2 compared to V1. Pro-inflammatory cytokines were not different between visits except for IL-6, which was significantly higher at V1 compared to V2 (P= 0.002).

Evidence of infection

The proportion of patients with increased CRP was higher at V1 compared to V2 (P= <0.001). Similarly, the number of patients with increased blood neutrophil count was higher at V1 compared to V2 (P= 0.001).

Symptoms score

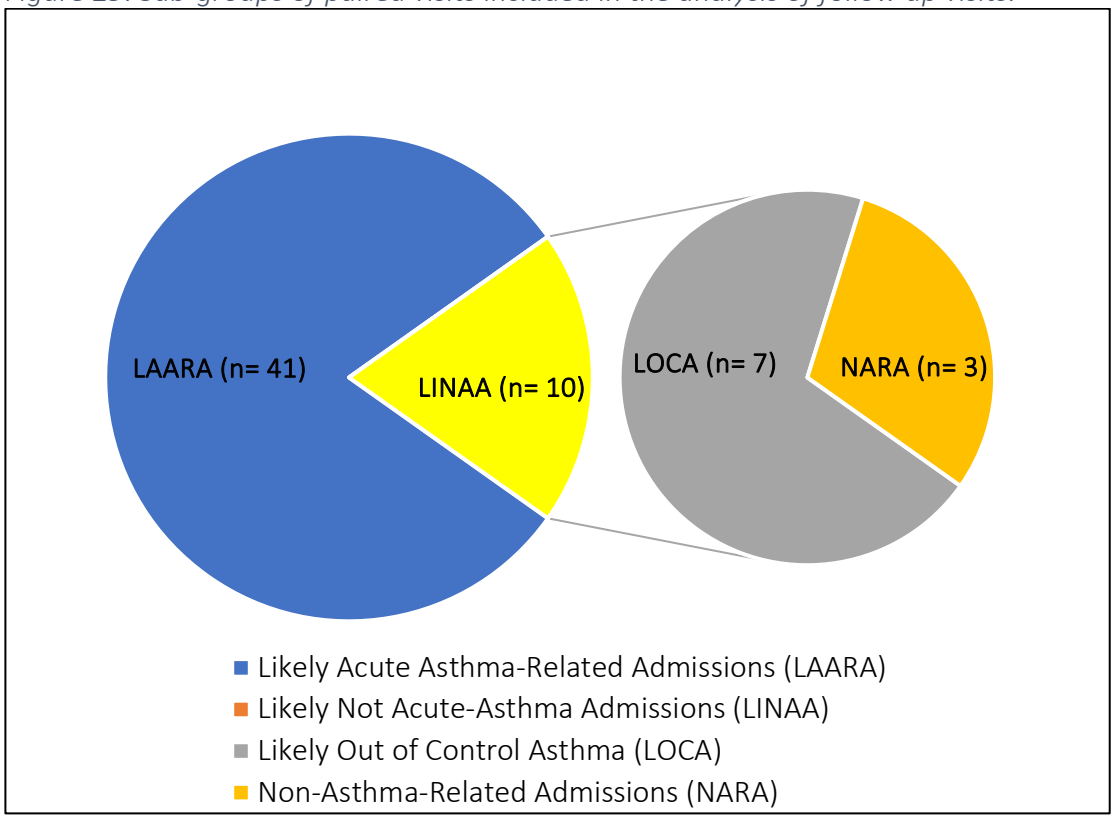
The overall AAQLQ score was significantly higher at V2 compared to V1 (P <0.001). The symptoms and emotional domains of the questionnaire were all significantly higher at V2 compared to V1 (P <0.001, P <0.001, respectively). The overall score, symptoms score, and emotional score of the AAQLQ were all significantly higher at V3 compared to V1 (P <0.001, P <0.001, P <0.001, respectively) but remained largely unchanged from V2 to V3. A summary of missing data is presented in Appendix L.

Sub-group analyses of paired admissions

Of the 60 patients who completed V2, 57 attended in person, of whom 51 had been included in the main comparative analysis at V1 (LAARA, n= 41 vs. LINAA, n= 10, Chapter 4); those (n= 51) will be included in the following sub-analyses of paired admissions. The following sub-analyses will describe and compare the clinical characteristics of paired admissions in the LAARA (n= 41), and LINAA (n= 10) groups (Figure 29). The

paired visits in the LOCA (n= 7) will be summarised and complete analysis included in Appendix M. Only 3 patients from the NARA completed V2 hence analysis of paired admission in the NARA group was not performed.

Figure 29. Sub-groups of paired visits included in the analysis of follow up visits.



Paired visits in the LAARA group (n= 41)

All the patients from LAARA group (n= 41) completed V2, of whom 38 attended in person, and 32 completed V3. The mean number of days between hospital discharge to V2 was 14.2 days (SD= 3.0).

Demographics

The majority of the patients who attended V2 were white and female with a mean age of 47.5 years (Table 51). A summary of missing data is presented in Appendix L.

Table 51. Demographic characteristics of patients in the LAARA group at V2.

Demographics (V2)	n= 41
	N (%) unless otherwise stated
Age (Mean ± SD) (years)	47.5 ± 16.0
Gender	
• *Male*	10 (24.4%)
• *Female*	31 (75.6%)
Ethnicity	
• *White/Caucasian*	30 (73.2%)
• *Black/Black British*	4 (9.8%)
• *Asian/Asian British*	5 (12.2%)
• *Other*	2 (4.9%)

Pulmonary traits at V1 compared to V2 in the LAARA group

Physiological parameters

Lung function parameters

Of those who attended V2 in person (n= 38), 37 completed spirometry at both visits. As expected, as this group had initially impaired lung function measures at V1, FEV_1% and FEV_1/FVC were all significantly higher at V2 compared to V1 (Table 52). The proportion of patients with impaired FEV_1% and FEV_1/FVC were similar at V2 compared to V1. Mean PEFR% and the proportion of patients with normal PEFR were significantly higher at V2 compared to V1 (Table 52). A summary of missing data is presented in Appendix L.

Table 52. Spirometry measures at V1 compared to V2 in the LAARA group.

Spirometry measures	LAARA V1 (n= 41)	LAARA V2 (n= 41)	P value
	N (%) unless otherwise stated		
FEV_1% (Mean ± SD) (n= 37)	59.4 ± 19.9	82.2 ± 17.5	**<0.001**
FVC% (Mean ± SD) (n= 37)	76.8 ± 17.2	92.2 ± 15.7	**<0.001**
FEV_1/FVC (Mean ± SD) (n= 37)	62.4 ± 15.0	72.6 ± 12.2	**<0.001**
PEFR% (Mean ± SD) (n= 37)	75.3 ± 24.5	100.9 ± 17.9	**<0.001**
FEV_1 <80%	31 of 37 (83.8)	18 of 37 (48.7)	0.09
FVC <80%	22 of 37 (59.5)	8 of 37 (21.6)	0.32
FEV_1 >80% and FVC <80%	0 of 37 (0)	0 of 37 (0)	
FEV_1/FVC <70%	28 of 37 (75.7)	13 of 37 (35.1)	0.57
PEFR <75%†	17 of 37 (45.9)	2 of 37 (5.4)	**0.01**

†Compared to best PEFR value or predicted PEFR if best is not available.

All the patients who attended V2 in person (n= 38) completed FOT at both visits. The mean R5 value was 6.1 cmH2O/L/s at V1 compared to 4.8 cmH2O/L/s at V2 (P <0.001) (Table 53). A summary of missing data is presented in Appendix L.

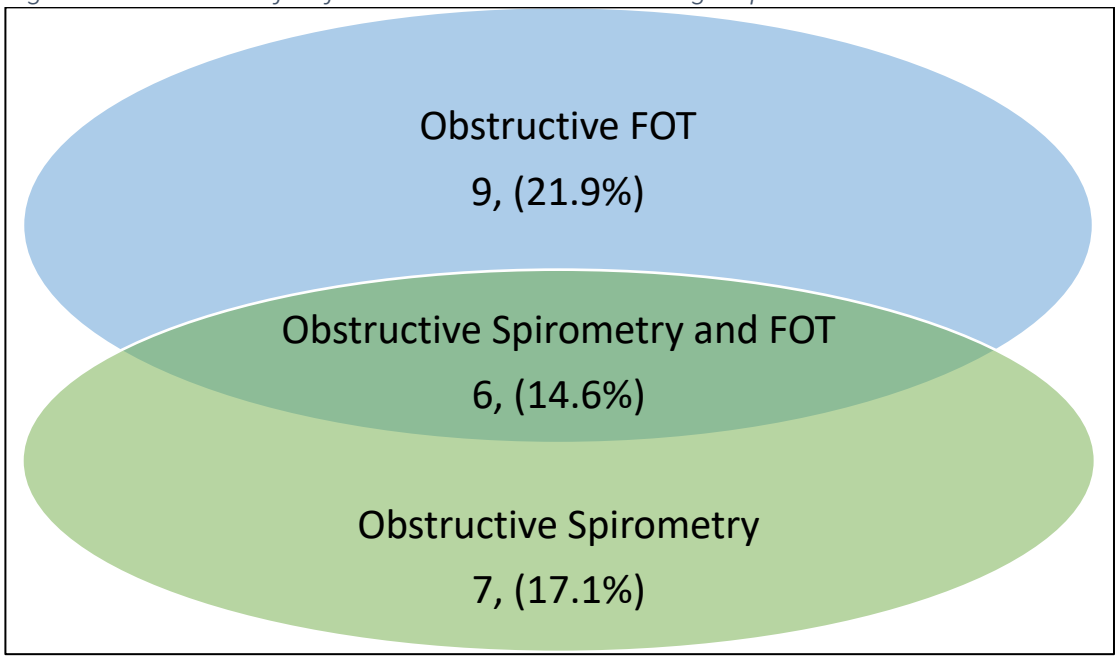
Table 53. Forced oscillation technique measures at V1 compared to V2 in the LAARA group.

FOT measures	LAARA V1 (n= 41)	LAARA V2 (n= 41)	P value
	N (%) unless otherwise stated		
R5 (Mean ± SD) (n= 38)	6.1 ± 2.4	4.8 ± 1.8	<0.001
R5 >5	25 of 38 (65.8)	15 of 38 (39.5)	0.89

Overall evidence of airflow limitation at V2 in the LAARA group

22 (53.7%) of the patients in the LAARA group had evidence of airflow limitation on either or both measures of airflow obstruction at V2 compared to V1 (P= 0.005). 9 (21.9%) of the patients had normal spirometry and obstructive FOT. 7 (17.1%) had normal FOT and obstructive spirometry. 6 (14.6%) had obstructive spirometry and obstructive FOT (Figure 30).

Figure 30. Evidence of airflow limitation in the LAARA group at V2.



Small airways disease

$FEF_{25-75\%}$ was significantly higher at V2 compared to V1 (Table 54). Similarly, R5-20 was significantly lower at V2 compared to V1. The proportion of patients with small airway disease, as per Lipworth's criteria, was higher at V2 compared to V1 (Table 54).

Table 54. Small airway measures at V1 compared to V2 in the LAARA group.

Small airway disease	LAARA V1 (n= 41)	LAARA V2 (n= 41)	P value
	N (%) unless otherwise stated		
$FEF_{25-75\%}$ (Mean ± SD) (n= 37)	38.6 ± 24.8	66.5 ± 27.3	**<0.001**
R5-R20 (Mean ± SD) (n= 38)	2.1 ± 1.6	1.2 ± 1.1	**0.008**
$FEF_{25-75\%}$ <65%	29 of 37 (78.4)	15 of 37 (40.5)	0.14
R5-R20 >1	28 of 38 (75.7)	18 of 38 (47.4)	0.25
Small airway disease*†	5 of 37 (13.5)	9 of 37 (24.3)	**<0.001**

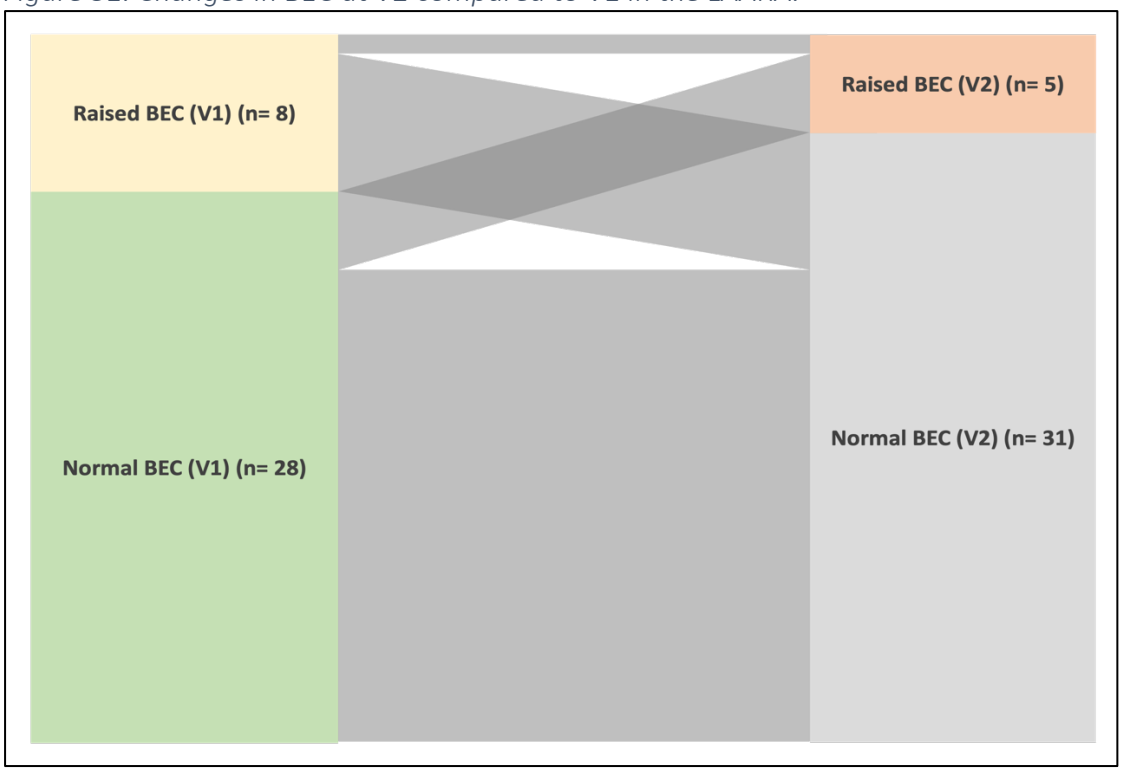
**Defined as per Lipworth's criteria: Preserved FEV_1 (>80%) and (FEF_{25-75} <60% OR R5-20 >1 CmH2O/L/s). †In those who completed both measure of airflow obstruction at both visits.*

Inflammatory profile at V1 compared to V2 in the LAARA group

Blood eosinophils

The median BEC was not different between the visits (Table 55). The proportion of patients with BEC ≥0.4 $x10^9$/L was higher at V1 compared to V2 (Figure 31).

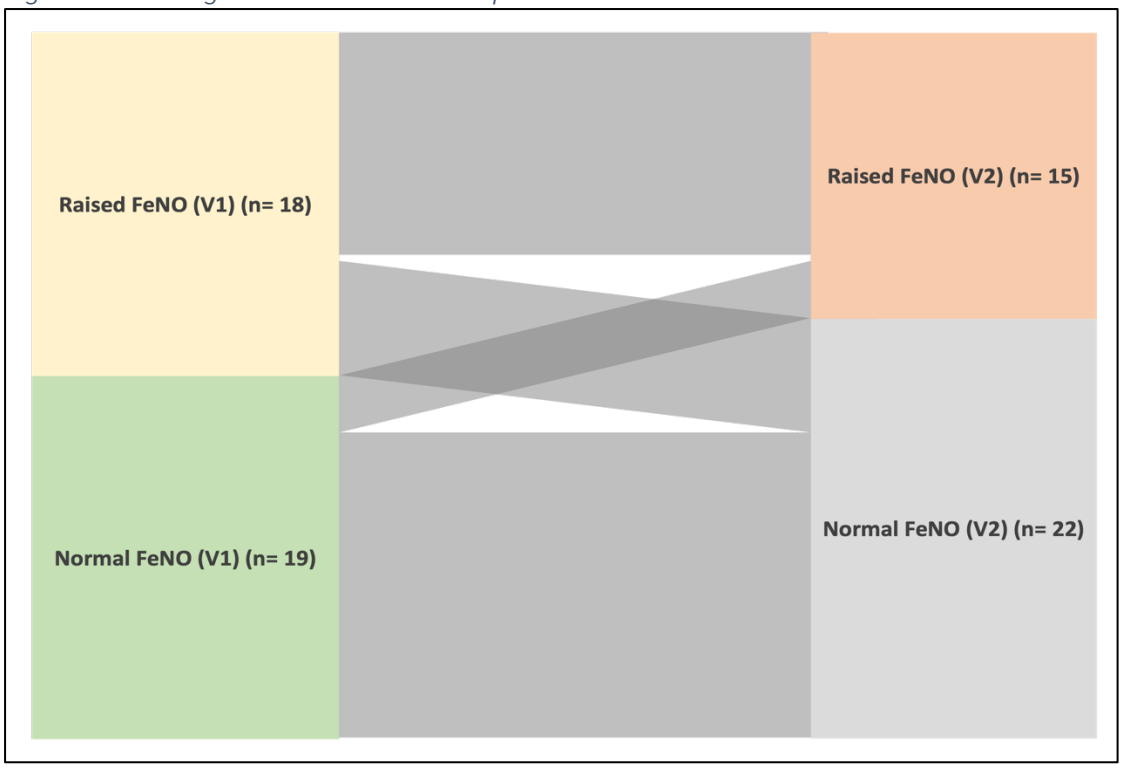
Figure 31. Changes in BEC at V2 compared to V1 in the LAARA.



FeNO

Median FeNO tended to be slightly increased at V1 (P= 0.11). The proportion of patients with FeNO ≥25 ppb was similar between visits (Table 55) (Figure 32). A summary of missing data is presented in Appendix L.

Figure 32. Changes in FeNO at V2 compared to V1 in the LAARA.



Sputum eosinophils

Sputum eosinophil% tended to be slightly decreased at V2 compared to V1 (P= 0.11) (Table 55). The proportion of patients with sputum eosinophils ≥3% and ≥2% was not different between the visits.

Sputum neutrophils

The median SNC% was significantly lower at V1 compared to V2 (P= 0.03). The proportion of patients with increased sputum neutrophils was not different between the visits.

T2-high inflammation

The proportion of patients with raised markers of $T2^{APEX3}$ inflammation was similar between visits (Table 55). 14 of 21 (66.7%) with increased $T2^{APEX3}$ at V1 and 4 of 17 (23.5%) with normal $T2^{APEX3}$ markers at V1 had increased $T2^{APEX3}$ markers at V2 (Figure 33).

Figure 33. Changes in T2 inflammation at V2 compared to V1 in the LAARA.

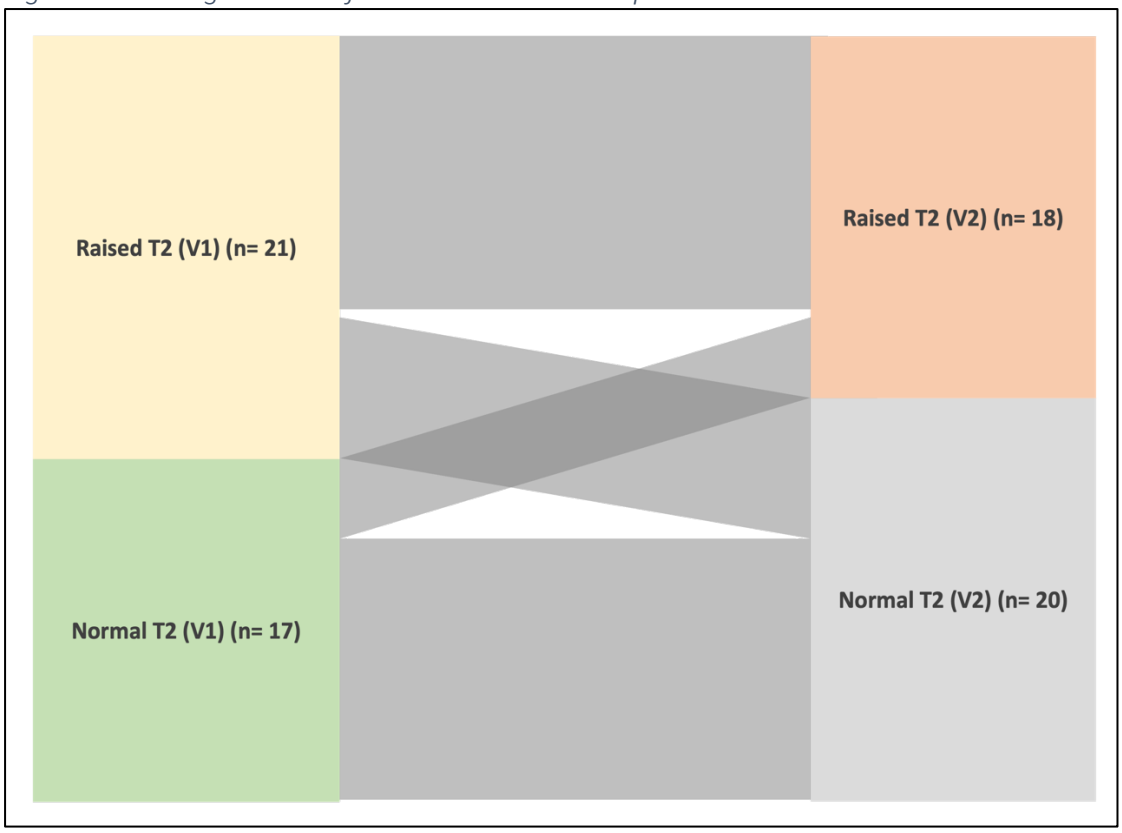


Table 55. Inflammatory biomarkers at V2 compared to V1 in the LAARA group.

Inflammatory measures	LAARA V1 (n= 41)	LAARA V2 (n= 41)	P value
	N (%) unless stated otherwise		
BEC Median (IQR) (n= 36)	0.15 (0.04- 0.35)	0.22 (0.09- 0.33)	0.58
FeNO Median (IQR) (n= 37)	21 (12- 67)	18 (12- 38)	0.11
SEC% Median (IQR) (n= 7)	3.0 (0- 23.9)	0.25 (0- 1.5)	0.11
SNC% Median (IQR) (n= 7)	66.3 (43.1- 72.4)	79.3 (75.6- 97.0)	**0.03**
SNC ≥65%	4 of 7 (57.1)	7 of 7 (100)	NA
Raised T2^{APEX3}	21 of 38 (55.3)	18 of 38 (47.4)	0.87
• BEC ≥0.4 x10^9/L	8 of 36 (22.2)	5 of 36 (13.9)	**<0.001**
• FeNO ≥25 ppb	18 of 37 (48.7)	15 of 37 (40.5)	0.53
• SEC ≥3%	4 of 7 (57.1)	1 of 7 (14.3)	NA
Raised T2^{GINA}	28 of 38 (73.7)	26 of 38 (68.4)	**0.01**
• BEC ≥0.15 x10^9/L	18 of 36 (50.0)	22 of 36 (61.1)	0.48
• FeNO ≥20 ppb	21 of 37 (56.8)	18 of 37 (48.7)	0.75
• SEC ≥2%	4 of 7 (57.1)	1 of 7 (14.3)	NA

Cytokine profiling

T2-high cytokines, epithelial derived cytokines, and Th17 cytokines were all similar between visits. Interferons were also similar at V2 compared to V1. IL-6, however, was significantly decreased at V2 compared to V1 (P= 0.01) (Table 56). A summary of missing data is presented in Appendix L.

Table 56. Cytokines profiling at V2 compared to V1 in the LAARA.

Cytokine profiles	LAARA V1 (n= 41)	LAARA V2 (n= 41)	P value
T2-high cytokines	Median (IQR)		
• IL-5 Median (IQR) (n= 6)	1.8 (1.6 – 3.0)	2.5 (2.2 – 10.0)	0.56
• IL-13 (Mean ± SD) (n= 18)	383.3 ± 298.6	458.4 ± 302.9	0.48
• IL-4 Median (IQR) (n= 24)	49.9 (35.7 – 68.9)	45.1 (27.7 – 75.0)	0.86
Epithelial derived cytokines			
• IL-33 Median (IQR) (n= 19)	96.5 (47.6 – 131.3)	56.6 (32.9 – 84.8)	0.10
• TSLP Median (IQR) (n= 24)	1.7 (1.2 – 2.2)	1.7 (1.1 – 2.6)	0.56
Th17 cytokines			
• IL-17 Median (IQR) (n= 24)	402.3 (277.0 – 502.5)	441.6 (359.9 – 805.5)	0.44
Pro-inflammatory cytokines			
IL-8 Median (IQR) (n= 21)	1695.9 (849.9 –2740.3)	1116.4 (780.1 – 3742.2)	1
• IL-1β Median (IQR) (n= 24)	41.1 (25.4 – 118.9)	63.1 (40.6 – 97.6)	0.85
• IL-6 Median (IQR) (n= 24)	17.4 (7.8 – 36.2)	8.5 (3.9 – 15.9)	**0.01**
• TNF-α Median (IQR) (n= 24)	14.2 (9.1 – 32.4)	18.1 (11.8 – 25.7)	0.66
Interferons			
• IFN-α Median (IQR) (n= 24)	10.6 (6.5 – 21.9)	17.6 (7.2 – 23.2)	0.77
• IFN-γ Median (IQR) (n= 24)	5.4 (3.9 – 7.6)	5.1 (2.7 – 7.6)	0.68
• IFN-β Median (IQR) (n= 12)	14.2 (5.0 – 17.5)	13.3 (9.6 – 30.2)	0.15

Evidence of infections

The number of patients with increased markers of infection was increased at V1 compared to V2 (Table 57).

Table 57. Measures of infection at V1 compared to V2 in the LAARA group.

Infection status	LAARA V1 (n=41)	LAARA V2 (n=41)	P value
	N (%)		
CRP (>10 mg/L)	14 of 38 (36.8)	7 of 38 (18.4)	0.02
BNC (>8 x10^9/L)	13 of 36 (36.1)	7 of 36 (19.4)	0.01

CRP: c-reactive protein. BNC: blood neutrophil count.

Symptoms score

The overall AAQLQ score was significantly higher at V2 compared to V1 (Table 58). Both the symptoms and emotional scores were higher at V2 compared to V1. A summary of missing data is presented in Appendix L.

Table 58. Acute Asthma Quality of Life Questionnaire at V1 compared to V2 in the LAARA group.

AAQLQ	LAARA V1 (n=41)	LAARA V2 (n=41)	P value
	N (%) unless stated otherwise		
AAQLQ overall score Median (IQR)	3.6 (3.0- 5.3)	6.4 (5.2- 6.8)	<0.001
Minimum important difference			
• *No difference between visits (<0.5)*	8 of 41 (19.5)		
• *Minimum difference (≥0.5)*	4 of 41 (9.8)		
• *Moderate difference (>1.0)*	3 of 41 (7.3)		
• *Large difference (>1.51)*	26 of 41 (63.4)		
Symptoms score Median (IQR)	3.5 (2.7- 5.2)	6.5 (5.5- 6.8)	<0.001
Minimum important difference			
• *No different between visits (<0.5)*	8 of 41 (19.5)		
• *Minimum difference (≥0.5)*	2 of 41 (4.9)		
• *Moderate difference (>1.0)*	7 of 41 (17.1)		
• *Large difference (>1.51)*	24 of 41 (58.5)		
Emotional score Median (IQR)	3.8 (2.8- 5.4)	6.2 (5.0- 6.8)	<0.001
Minimum important difference			
• *No different between visits (<0.5)*	10 of 41 (24.4)		
• *Minimum difference (≥0.5)*	4 of 41 (9.8)		
• *Moderate difference (>1.0)*	6 of 41 (14.6)		
• *Large difference (>1.51)*	21 of 41 (51.2)		

The AAQLQ scores were significantly higher at V3 compared to V1 but remained largely unchanged from V2 to V3 (Table 59).

Table 59. Acute Asthma Quality of Life Questionnaire at V3 compared to V2 and V1 in the LAARA.

AAQLQ	LAARA V1 (n=32)	LAARA V3 (n= 32)	P value	LAARA V2 (n=32)	LAARA V3 (n= 32)	P value
	Median (IQR)			Median (IQR)		
Overall score†	3.5 (2.9-5.1)	6.4 (5.4-6.7)	<0.001	6.5 (5.2-6.8)	6.4 (5.4-6.7)	0.80
Symptoms score†	3.5 (2.7-4.9)	6.4 (5.9-7.0)	<0.001	6.7 (5.5-7.0)	6.4 (5.9-7.0)	0.64
Emotional score†	3.8 (2.8-5.0)	6.4 (5.4-6.6)	<0.001	6.2 (5.3-6.9)	6.4 (5.4-6.6)	0.99

†In those who completed V3.

Paired visits in the LINAA group (n= 10)

All patients from the LINAA group (n= 10) attended V2 in person and 8 completed V3. The mean number of days between hospital discharge to V2 in the LINAA group is 13 days (SD= 1.6). A summary of missing data is presented in Appendix L.

Demographics

The majority of the patients who attended V2 were white and female with a mean age of 38.2 years (Table 60).

Table 60. Demographic characteristics of patients in the LINAA group at V2.

Demographics (V2)	n= 10
	N (%) unless otherwise stated
Age (Mean ± SD) (years)	38.2 ± 16.7
Gender	
• *Male*	4 (40)
• *Female*	6 (60)
Ethnicity	
• *White/Caucasian*	8 (80)
• *Black/Black British*	1 (10)
• *Asian/Asian British*	1 (10)
• *Other*	0 (0)

Pulmonary traits at V1 compared to V2 in the LINAA group

Physiological parameters

Lung function parameters

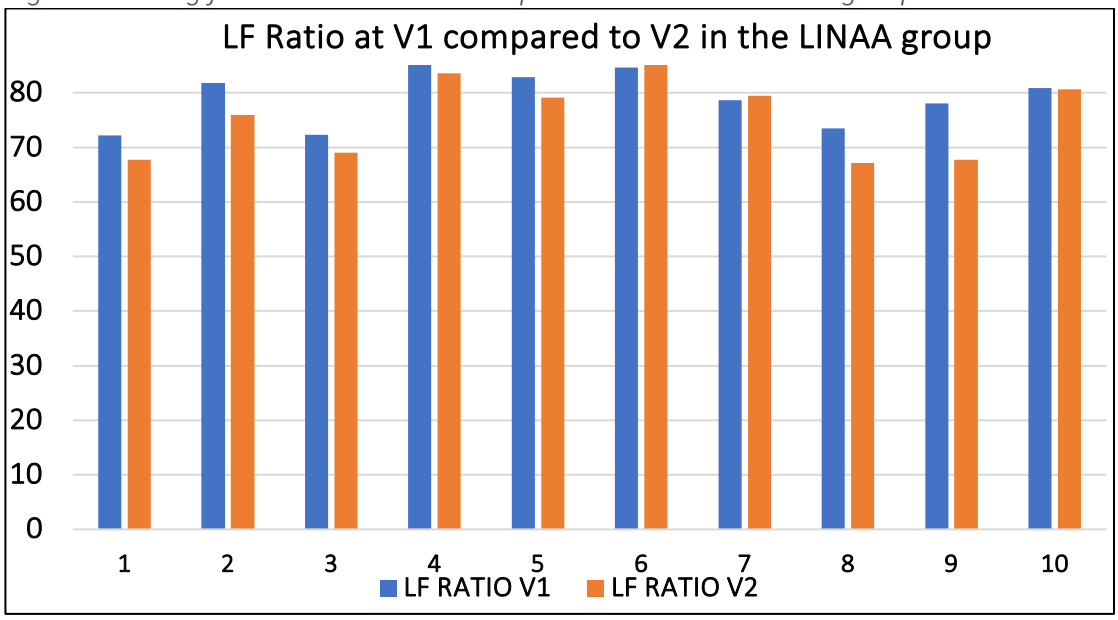
The mean FEV_1% was similar between the visits. FEV_1/FVC was significantly reduced at V2 compared to V1 (Figure 34) (Table 61). The proportion of those with reduced ratio was increased at V2 compared to V1 (40% vs. 0%, P= 0.04). Mean PEFR% was similar at V1 compared to V2 (Table 61). A summary of missing data is presented in Appendix L.

Table 61. Spirometry measures at V1 compared to V2 in the LINAA group.

Spirometry measures	LINAA V1 (n= 10)	LINAA V2 (n= 10)	P value
	N (%) unless otherwise stated		
FEV_1% (Mean ± SD) (n= 10)	90.5 ± 6.8	89.0 ± 11.2	0.57
FVC% (Mean ± SD) (n= 10)	94.3 ± 5.9	96.8 ± 8.6	0.20
FEV_1/FVC (Mean ± SD) (n= 10)	78.9 ± 4.9	75.6 ± 7.1	**0.02**
PEFR% (Mean ± SD) (n= 10)	100.1 ± 8.4	99.1 ± 19.9	0.83
FEV_1 <80%	0 (0)	1 (10)	NA
FVC <80%	1 (10)	0 (0)	NA
FEV_1 >80% and FVC <80%	1 (10)	0 (0)	NA
FEV_1/FVC <70%	0 (0)	4 (40)	NA
PEFR <75%†	0 (0)	1 (10)	NA

†Compared to best PEFR value or predicted PEFR if best is not available.

Figure 34. Lung function ratio at V1 compared to V2 in the LINAA group.

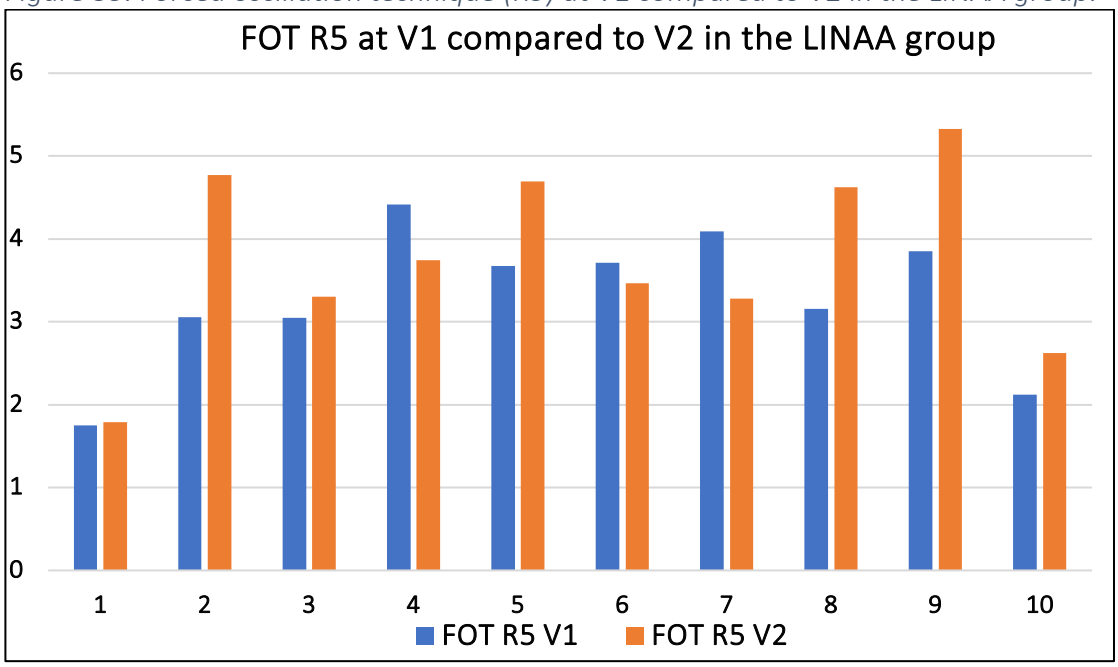


The mean R5 value was similar at V1 compared to V2 (Table 62) (Figure 35).

Table 62. Forced oscillation technique measures at V1 compared to V2 in the LINAA group.

FOT measures	LINAA V1 (n= 10)	LINAA V2 (n= 10)	P value
R5 (Mean ± SD) (n= 10)	3.29 ± 0.84	3.76 ± 1.10	0.13
R5 >5	0 (0%)	1 (10%)	NA

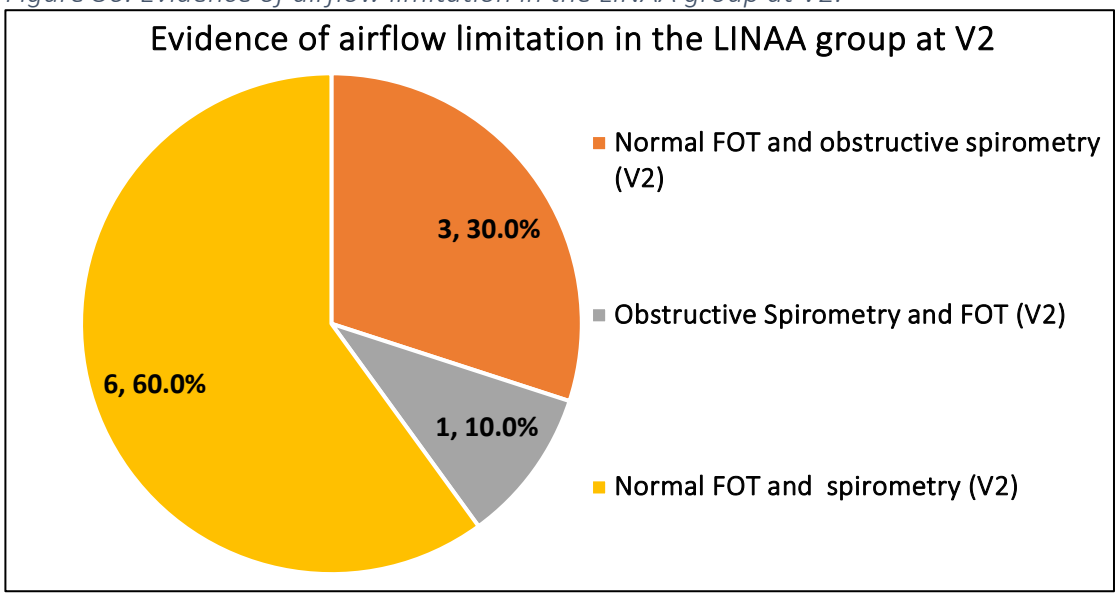
Figure 35. Forced oscillation technique (R5) at V1 compared to V2 in the LINAA group.



Overall evidence of airflow limitation at V2 in the LINAA group

Compared to V1, 4 (40%) of the patients in the LINAA group had evidence of airflow limitation at V2 (P= 0.04). 3 (30%) had normal FOT and obstructive spirometry, and 1 (10%) had obstructive spirometry and FOT (Figure 36).

Figure 36. Evidence of airflow limitation in the LINAA group at V2.



Small airways disease

Mean $FEF_{25-75\%}$ tended to be higher at V1 compared to V2 although this was not significantly different (Table 63). Median R5-20 was similar between the visits. The proportion of patients with small airway disease, as per Lipworth's criteria, was similar between the visits. A summary of missing data is presented in Appendix L.

Table 63. Small airway measures at V1 compared to V2 in the LINAA group.

Small airway disease	LINAA V1 (n= 10)	LINAA V2 (n= 10)	P value
	N (%) unless stated otherwise		
$FEF_{25-75\%}$ (Mean ± SD) (n= 10)	82.9 ± 15.7	73.4 ± 23.9	0.09
R5-R20 Median (IQR) (n= 10)	0.4 (0.2- 0.7)	0.8 (0.3- 1.1)	0.38
$FEF_{25-75\%}$ <65%	1 (10)	3 (30)	NA
R5-R20 >1	2 (20)	3 (30)	NA
Small airway disease*†	2 (20)	4 (40)	NA

**Defined as per Lipworth's criteria: Preserved FEV_1 (>80%) and (FEF_{25-75} <60% OR R5-20 >1 CmH2O/L/s). †In those who completed both measure of airflow obstruction at both visits.*

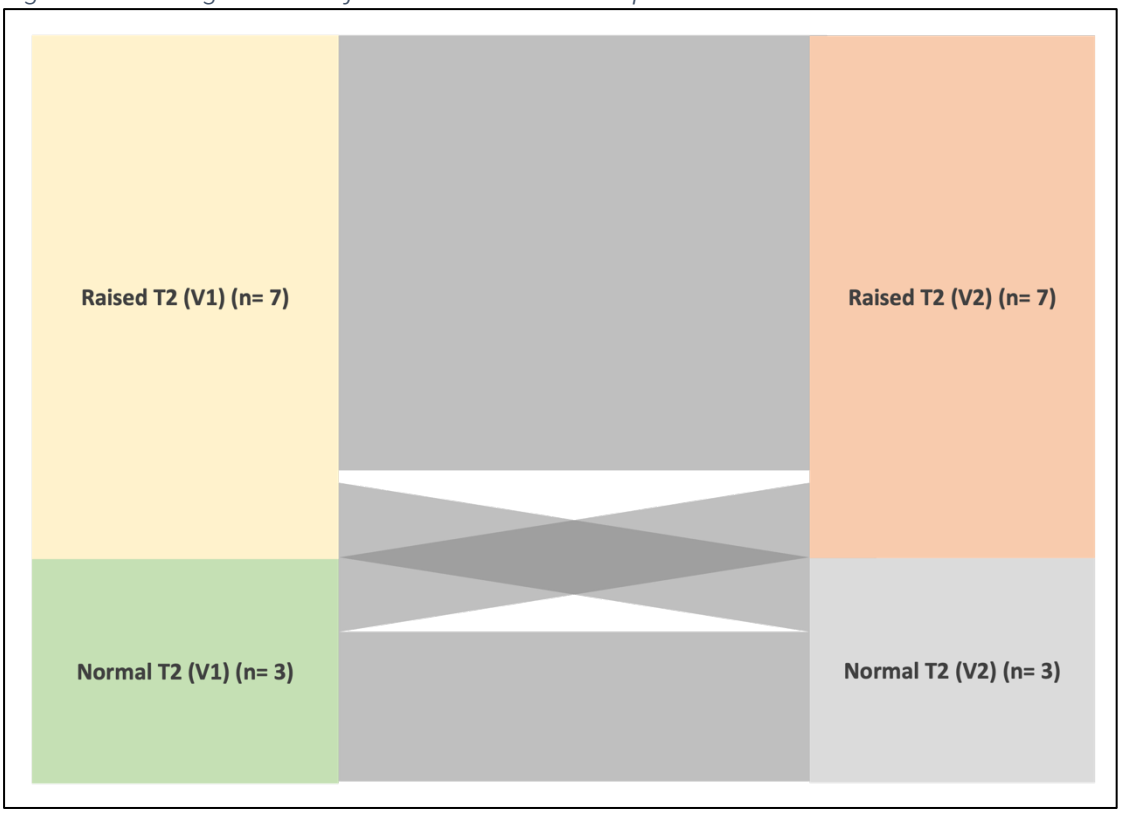
Inflammatory profile at V1 compared to V2 in the LINAA group

Both BEC and FeNO were similar between the visits (Table 64). Difference in sputum markers was not assessed due to the limited number of paired samples obtained. The proportion of patients with increased markers of T2 inflammation was not different between visits (Table 64). 6 of 7 (85.7%) patients with increased $T2^{APEX3}$ markers at V1 still had increased $T2^{APEX3}$ markers at V2 (Figure 37).

Table 64. Inflammatory biomarkers at V1 compared to V2 in the LINAA group.

Inflammatory measures	LINAA V1 (n= 10)	LINAA V2 (n= 10)	P value
	N (%) unless stated otherwise		
BEC Median (IQR) (n= 10)	0.3 (0.01- 0.5)	0.2 (0.1- 0.5)	0.41
FeNO Median (IQR) (n= 10)	39.5 (14- 78)	30.5 (13- 51)	0.19
SEC% Median (IQR) (n= 1)	20.5 (NA)	5 (NA)	NA
SNC% Median (IQR) (n= 1)	70.8 (NA)	61 (NA)	NA
SNC ≥65%	1 of 1 (100)	0 of 1 (0)	NA
Raised $T2^{APEX3}$	7 (70)	7 (70)	NA
BEC ≥0.4 $x10^9$/L	3 (30)	4 (40)	NA
FeNO ≥25 ppb	7 (70)	7 (70)	NA
SEC ≥3%	1 of 1 (100)	1 of 1 (100)	NA
Raised $T2^{GINA}$	8 (80)	8 (80)	NA
BEC ≥0.15 $x10^9$/L	7 (70)	6 (60)	NA
FeNO ≥20 ppb	7 (70)	7 (70)	NA
SEC ≥2%	1 of 1 (100)	1 of 1 (100)	NA

Figure 37. Changes in T2 inflammation at V2 compared to V1 in the LINAA.



Cytokines of T2-high asthma, epithelial derived cytokines, Th17 cytokines, and interferons were all similar between visits. IL-6 was significantly decreased at V2 compared to V1 (Table 65).

Table 65. Cytokines profiling at V2 compared to V1 in the LINAA.

Cytokine profiles	LINAA V1 (n= 10)	LINAA V2 (n= 10)	P value
T2-high cytokines	Median (IQR)		
• IL-5 Median (IQR) (n= 3)	1.6 (1.5 – 1.8)	0.8 (0.5 – 3.7)	1
• IL-13 (Mean ± SD) (n= 5)	93.6 (44.9 – 117.3)	454.6 (375.6 – 496.9)	0.71
• IL-4 Median (IQR) (n= 9)	32.3 (29.4 – 38.5)	47.8 (35.1 – 50.4)	0.44
Epithelial derived cytokines			
• IL-33 Median (IQR) (n= 8)	84.9 (52.4 – 126.6)	93.3 (54.7 – 110.9)	0.64
• TSLP Median (IQR) (n= 9)	1.1 (0.7 – 3.8)	1.4 (0.6 – 1.8)	0.72
Th17 cytokines			
• IL-17 Median (IQR) (n= 9)	395.6 (330.4 – 466.3)	398.6 (323.1 – 459.3)	0.82
Pro-inflammatory cytokines			
IL-8 Median (IQR) (n= 8)	2488.8 (786.0 –3695.7)	817.3 (732.7 – 1282.9)	0.54
• IL-1β Median (IQR) (n= 9)	73.2 (41.6 – 124.6)	37.3 (26.8 – 109.2)	0.16
• IL-6 Median (IQR) (n= 9)	24.5 (7.8 – 32.2)	4.9 (3.3 – 6.4)	**0.004**
• TNF-α Median (IQR) (n= 9)	12.6 (7.3 – 22.6)	15.5 (14.6 – 17.1)	1
Interferons			
• IFN-α Median (IQR) (n= 9)	7.0 (6.6 – 21.8)	11.8 (9.9 – 14.1)	0.57
• IFN-γ Median (IQR) (n= 9)	3.4 (1.9 – 14.8)	4.5 (3.4 – 6.7)	0.30
• IFN-β Median (IQR) (n= 3)	4.0 (2.6 – 26.8)	13.3 (12.2 – 14.9)	1

Evidence of infections

The proportion of patients with increased CRP and BNC were similar at V1 compared to V2 (Table 66).

Table 66. Measures of infection at V1 compared to V2 in the LINAA group.

Infection status	LINAA V1 (n= 10)	LINAA V2 (n= 10)	P value
	N (%)		
CRP (> 10 mg/L) (n= 10)	2 (20)	2 (20)	NA
BNC (> 8 x10^9/L) (n= 10)	3 (30)	3 (30)	NA

CRP: c-reactive protein. BNC: blood neutrophil count.

Symptoms score

The overall AAQLQ score was significantly higher at V2 compared to V1 (Table 67). The symptoms domain of the questionnaire was significantly higher at V2 compared to V1.

Table 67. Acute Asthma Quality of Life Questionnaire at V1 compared to V2 in the LINAA group.

AAQLQ score	LINAA V1 (n= 10)	LINAA V2 (n= 10)	P value
	N (%) unless stated otherwise		
AAQLQ overall score Median (IQR)	3.8 (3.6- 4.9)	5.5 (4.7- 6.5)	0.02
Minimum important difference			
• *No difference between visits (<0.5)*	3 (30)		
• *Minimum difference (≥0.5)*	3 (30)		
• *Moderate difference (>1.0)*	1 (10)		
• *Large difference (>1.51)*	3 (30)		
Symptoms score Median (IQR)	4.4 (3.7- 5.2)	6 (4.6- 6.7)	0.04
Minimum important difference			
• *No different between visits (<0.5)*	3 (30)		
• *Minimum difference (≥0.5)*	2 (20)		
• *Moderate difference (>1.0)*	2 (20)		
• *Large difference (>1.51)*	3 (30)		
Emotional score Median (IQR)	3.2 (2.4- 4.9)	4.8 (4.1- 6.6)	0.06
Minimum important difference			
• *No different between visits (<0.5)*	5 (50)		
• *Minimum difference (≥0.5)*	0 (0)		
• *Moderate difference (>1.0)*	2 (20)		
• *Large difference (>1.51)*	3 (30)		

Scores of the AAQLQ were significantly higher at V3 compared to V1 but remained largely unchanged from V2 to V3 (Table 68). A summary of missing data is presented in Appendix L.

Table 68. Acute Asthma Quality of Life Questionnaire at V3 compared to V2 and V1 in the LINAA.

AAQLQ	LINAA V1 (n= 8)	LINAA V3 (n= 8)	P value	LINAA V2 (n= 8)	LINAA V3 (n= 8)	P value
	Median (IQR)			Median (IQR)		
Overall score†	3.8 (3.6-4.6)	6.4 (5.4-6.5)	0.02	6.2 (5.0-6.6)	6.4 (5.4-6.5)	0.41
Symptoms score†	4.4 (3.- 5.3)	6.2 (6.0-6.3)	0.02	6.3 (5.8-6.8)	6.2 (6.0-6.3)	1
Emotional score†	3.2 (2.8-4.5)	6.6 (5.0-6.9)	0.04	5.6 (4.2-6.6)	6.6 (5.0-6.9)	0.40

†In those who completed V3.

Summary of Appendix M: APEX3- Paired visits in the LOCA group (n= 7)

Paired visits in the LOCA group were analysed using the same methodology used in the LAARA and the LINAA groups. Due to the low number of patients, the LOCA group analysis will be briefly summarised, and the complete analysis included in Appendix M. 7 patients from the LOCA group attended V2. The median length of time from hospital discharge to V2 in the LOCA group was 13 days (IQR= 12.0 – 14.0). Lung function measures were largely unchanged from V1 to V2 except for lung function ratio being slightly decreased at V2 compared to V1 (78.0 vs. 68.9, P= 0.02). Markers of inflammation and infection were similar at V2 compared to V1. Symptom scores were not different between the visits.

Changes in lung function, inflammation, and symptom scores between visits in the Likely acute asthma-related admissions (LAARA, n=41) vs. Likely not acute-asthma admissions (LINAA, n=10)

Lung function measures

As expected, as sub-groups were defined based on the presence or absence of airflow limitation at V1, between visit improvement in lung function measures was significantly higher in the LAARA compared to the LINAA (Table 69). The proportion of patients who achieved a minimum clinically important different in FEV_1% was increased in the LAARA

compared to the LINAA (P= 0.001). Changes in measures of small airways dysfunction were also significantly higher in the LAARA compared to the LINAA.

Table 69. Changes in lung function parameters between visits in the LAARA compared to the LINAA.

Lung function measures	LAARA (n= 41)	LINAA (n= 10)	P value
	N (%) unless stated otherwise		
ΔFEV_1% (Mean ± SD)	22.8 ± 19.5	-1.5 ± 7.8	**<0.001**
ΔFEV_1% MCID*†	25 of 37 (67.6)	1 of 10 (10)	**0.001**
ΔFEV_1/FVC Median (IQR)	10.4 (2.5- 16.2)	-3.5 (-5.5--0.7)	**<0.001**
ΔPEFR% (Mean ± SD)	25.6 ± 23.2	-1.0 ± 14.9	**<0.001**
ΔR5 Median (IQR)	-0.95 (-2.2--0.01)	0.38 (-0.2- 1.4)	**0.001**
$\Delta FEF_{25-75\%}$ Median (IQR)	27.2 (7.2- 43.1)	-6.7 (-20.9- 2.3)	**<0.001**
ΔR5-R20 Median (IQR)	-0.51 (-1.4- 0.2)	0.19 (-0.2 – 0.6)	**0.01**

**A minimum of 20% change compared to V1. †In those who completed spirometry at both visits.*

Predicting a minimum clinically important improvement in FEV_1%

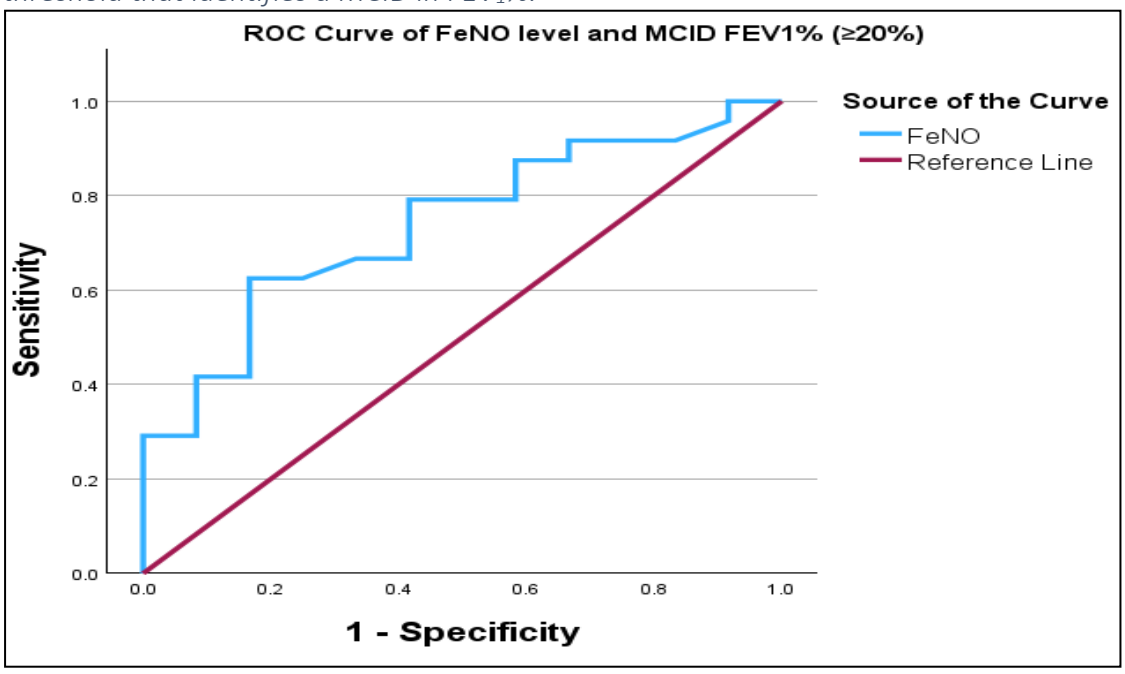
Using a multivariate logistic model, PEFR <75% (at V1) and FeNO ≥25 ppb (at V1) were independent predictors of a minimal clinically important improvement in FEV_1% (≥20% improvement) (Table 70). The optimal FeNO cutoff (at V1) for the identification of a FEV_1% MCID is 24.5 ppb with a sensitivity of 63% and a specificity of 83% (AUC= 0.74, P= 0.005, PPV= 88.5%, NPV= 51.8%) (Figure 38).

Table 70. Multivariate logistic regression model analysing factors predicting minimal clinically important improvement in FEV_1%.

	Unadjusted OR	95% CI	P value	Adjusted OR	95% CI	P value
BEC ≥ 0.15	3.8	0.9 – 20.5	0.08			
BEC ≥ 0.4*	81634441	7.149913e-77 – NA	0.99			
FeNO ≥25 ppb	8.3	1.7 – 62.9	**0.01**	7.1	1.2 – 62.4	**0.04**
FeNO >50 ppb	4.2	0.9 – 31.5	0.09			
SEC ≥3%*	440112421	8.951414e-134 – NA	0.99			
PEFR <75%	19.5	3.1 – 388.4	**0.008**	16.0	2.2 – 337.8	**0.01**
Wheezing	2.4	0.6 – 10.8	0.21			
AAQLQ symptoms	1.0	0.67 – 1.6	0.94			

**Too few observations to calculate an odds ratio.*

Figure 38. Receiver-operating characteristic curve to determine the optimal FeNO threshold that identifies a MCID in FEV_1%.



Inflammatory biomarkers

Improvement in inflammatory markers was similar between the groups (Table 71). Improvement in sputum eosinophils was not assessed due to the limited number of sputum samples obtained.

Table 71. Changes in inflammatory biomarkers between visits in the LAARA compared to the LINAA.

Inflammatory biomarkers	LAARA (n= 41)	LINAA (n= 10)	P value
	N (%) unless stated otherwise		
ΔBEC (Mean ± SD)	-0.03 ± 0.33	0.03 ± 0.11	0.37
ΔFeNO (Mean ± SD)	-8.30 ± 45.61	-16.50 ± 36.60	0.55
ΔFeNO MCID*	33 of 37 (89.19)	8 of 10 (80)	0.59

**A minimum of 20% change compared to V1.*

Symptoms score

The overall AAQLQ score improvement was slightly higher in the LAARA compared to the LINAA. The symptoms score improvement was significantly higher in the LAARA group (Table 72). A summary of missing data is presented in Appendix L.

Table 72. Changes in symptoms scores between visits in the LAARA compared to the LINAA.

AAQLQ score	LAARA (n= 41)	LINAA (n= 10)	P value
	Mean ± SD		
ΔOverall score	1.95 ± 1.57	1.08 ± 1.18	0.06
ΔSymptoms score	2.11 ± 1.69	0.95 ± 1.28	**0.02**
ΔEmotional score	1.75 ± 1.64	1.24 ± 1.50	0.36

Changes in lung function and symptom scores between visits in patients with T2-low (n= 24) compared to T2-high (n= 36) asthma attacks as per APEX3 criteria for T2-high inflammation (FeNO level ≥25 ppb, blood eosinophil count of ≥0.4 x10^9/L, or sputum eosinophil count of ≥3%)

Lung function measures

Between visit FEV_1% and PEFR% improvement were significantly higher in the T2-high AAs compared to the T2-low AAs. The proportion of patients who achieved a minimum clinically important difference in FEV_1% was higher in the T2-high AAs (p= 0.03). Mean lung function ratio improvement tended to be higher in the T2-high group (P= 0.05).

Improvement in R5 and measures of small airway disease was not different between the groups (Table 73). A summary of missing data is presented in Appendix L.

Table 73. Changes in lung function parameters between visits in patients with initially T2-high asthma attacks compared to T2-low asthma attacks.

Lung function measures	T2-low (n= 24)	T2-high (n= 36)	P value
	N (%) unless stated otherwise		
ΔFEV_1% (Mean ± SD)	9.3 ± 12.9	23.8 ± 22.6	**0.008**
ΔFEV_1% MCID*†	7 of 20 (35.0)	19 of 27 (70.4)	**0.03**
$\Delta FEV_1/FVC$ (Mean ± SD)	4.0 ± 6.5	9.9 ± 12.7	0.05
ΔPEFR% (Mean ± SD)	11.6 ± 14.6	26.1 ± 28.0	**0.03**
ΔR5 (Mean ± SD)	-0.6 ± 1.5	-1.1 ± 2.1	0.30
$\Delta FEF_{25-75\%}$ (Mean ± SD)	16.7 ± 21.9	22.2 ± 32.5	0.49
ΔR5-R20 (Mean ± SD)	-0.31 ± 1.2	-0.85 ± 1.5	0.15

**A minimum of 20% change compared to V1. †In those who completed spirometry at both visits.*

Symptoms score

AAQLQ scores were not different between the groups (Table 74). A summary of missing data is presented in Appendix L.

Table 74. Changes in AAQLQ scores between visits in patients with initially T2-high asthma attacks compared to T2-low asthma attacks.

AAQLQ scores	T2-low (n= 24)	T2-high (n= 36)	P value
ΔOverall score (Mean ± SD)	1.5 ± 1.3	1.9 ± 1.7	0.36
ΔSymptoms score (Mean ± SD)	1.7 ± 1.4	1.9 ± 1.8	0.57
ΔEmotional score Median (IQR)	1.2 (0.2- 2.3)	1.6 (0.4- 2.8)	0.23

Discussion

Further supporting the findings from the Audit and Chapter 3, the present study found that the group of patients with no evidence of airflow limitation at the time of hospitalisation for presumed asthma had no lung function improvement 2 weeks following hospital discharge and treatment with systemic corticosteroids. Further, a discrepancy between measures of lung physiology, spirometry and FOT has also been noted, likely due to inherent differences between the two techniques. Measures of small airways dysfunction significantly improved at V2 compared to V1 suggesting a possible contribution of small airway pathology in the pathophysiology of AAs.

Those with a T2-high phenotype demonstrated a significantly greater lung function improvement than those with T2-low exacerbations suggesting inflammatory phenotyping of AAs may be useful in predicting treatment response and reinforcing the need for a precision medicine approach to managing acute asthma episodes. Symptom improvement was not different between groups, highlighting the limitations of traditional symptom-based treatment algorithms. Importantly, a substantial number of patients demonstrated ongoing T2-high activity despite symptoms and lung function improvement, identifying a poorly understood population at potential higher risk of future exacerbation who may benefit from a more aggressive secondary prevention approach who warrant further investigation in future studies.

Lung function, inflammation, and symptoms improvement at recovery

Further supporting the findings of the Audit and those from V1 of the present study (Chapter 3), the group of patients with no evidence of airflow limitation at V1 (LINAA) had largely unchanged lung function at V2. Although a limited number of studies have addressed acute asthma misdiagnosis[493,494], this study is the most comprehensive investigation of this issue to date. The extent of lung function recovery following hospitalisation and treatment reported in the present study is novel and has not been previously reported. The closest comparable finding is from a recent study by Couillard et al.[475] (PRISMA), which demonstrated that a subgroup of patients with T2-Low/Low (BEC <0.15 x10^9/L and FeNO <25 ppb) asthma attacks had non-significant FEV_1% improvement seven days following treatment with OCS. Notably, baseline (post-bronchodilator) FEV_1% (91.7%) and FEV_1/FVC (76.8%) in the T2-Low/Low group in the

PRISMA study were similar to those observed at V1 in the LINAA group in the present study (90.5%, 78.9%, respectively).

However, while the PRISMA study reported a non-significant improvement in FEV_1% between visits, the present study found a non-significant reduction. This discrepancy can be attributed to differences in patient selection criteria: the PRISMA study included patients with a history of objectively confirmed asthma, whereas the present study relied on the attending physician's diagnosis of an asthma attack, irrespective of prior confirmation of asthma. Given that the T2-Low/Low group had a well-established asthma diagnosis, and their pre-bronchodilator FEV_1% was normal (88.1%) at V1 while symptom scores substantially improved at V2, it is likely that their acute episodes were symptom-predominant, potentially reflecting poorly controlled asthma (although non-asthma causes of symptoms worsening cannot be entirely excluded as those with a T2-Low/Low phenotype had greater symptoms suggestive of dysfunctional breathing), as supported by the slight improvement in lung function following treatment and existing evidence that poor asthma control is characterised by highly symptomatic periods with variable but largely preserved lung function and high bronchodilator responsiveness[155,156,667]. In contrast, the reduction in FEV_1% and increase in resistance (R5) between visits in the LINAA group may be attributed to the effect of bronchodilation prior to study recruitment. Previous studies have demonstrated that even healthy individuals can have a significant FEV_1% and R5 bronchodilator response[668]. The fact that symptom scores improved at V2 despite no improvement in lung function suggests that the symptom worsening observed on admission in the LINAA group may have been due to non-acute-asthmatic causes, or potentially non-asthma-related pathologies (as some of the patients in the LINAA had normal inflammatory markers), where corticosteroid responsiveness is diminished.

Furthermore, the increased number of patients with an impaired lung function ratio at V2 compared to V1 in the present study is likely explained by the reduction in FEV_1% at V2 compared to V1 and may reflect the limitations of using a fixed FEV_1/FVC cutoff, which is known to overestimate the degree airflow limitation[628]. While PRISMA reported findings comparable to ours, it is important to note that PRISMA aimed to assess the response to oral corticosteroids and evaluated patients seven days following

treatment. In contrast, the present study aimed to assess the degree of recovery, with patients followed up 14 days after hospital discharge.

Similar to findings from V1 (Chapter 3), a discrepancy between spirometry and FOT was observed, likely due to differences in the sensitivities of these measures in detecting airway abnormalities. About 19% of the patients who performed both spirometry and FOT at both visits had isolated obstructive R5. Given that FOT is more sensitive than spirometry in detecting airway abnormalities[668], a plausible explanation for this finding is the involvement of small airway abnormalities that are detected by FOT but not spirometry. This is supported by the slightly increased R5-R20 (1.1 cmH2O/L/s) at V2 and the increased number of patients meeting criteria for small airway disease at V2 compared to V1 (using Lipworth's criteria: preserved FEV_1% (>80%) and (FEF_{25-75} <60% OR R5-20 >1 cmH2O/L/s), which is likely secondary to the increased number of patients with normal FEV_1% at V2 compared to V1. 21.3% of the patients had isolated obstructive spirometry. Those are likely to have a normal FEV_1/FVC that is below the fixed cutoff used to identify airflow obstruction in the present study, as supported by their normal R5 values. The 15% of the patients with both R5 and FEV_1/FVC limitation may reflect a group of patients with a combination of small airway disease and a lung function ratio below 0.7. Although a relapse, or an impending relapse as patients who required asthma-related healthcare after V1 were excluded from V2, cannot be entirely excluded, it is unlikely given the significant improvement in symptoms between visits.

Results from visit 1 (Chapter 3) demonstrated significant small airway impairment. However, whether this observed impairment was due to a background of chronic small airway dysfunction or an acute, transient impairment could not be determined until data from visit two were available. Results from the follow-up visit suggest that the lung periphery is significantly affected during acute episodes of asthma, as measures of small airway function were found to be significantly improved at V2 compared to V1. Although $FEF_{25-75\%}$ is highly influenced by other spirometric measures[627] and increases in $FEF_{25-75\%}$ can arguably be related to FEV_1% improvement, the fact that R5-R20 also improved at V2 strongly suggests the involvement of the small airways. Thompson et al.[619] assessed acinar and conductive ventilation heterogeneity using the multiple-breath nitrogen washout test in hospitalised compared to stable asthma patients and reported increased conductive ventilation heterogeneity in exacerbating patients. Both

acinar and conductive ventilation heterogeneity markedly improved at follow-up, 4 weeks after hospital discharge. Although the role of the small airways in AAs remains poorly understood, results from the present study support a growing recognition that the small airways play an important role in the pathophysiology of asthma.

Although the majority of cytokines measured did not differ between V1 and V2, IL-6 levels were significantly lower at V2 across several comparison groups, including the Likely Not Acute Asthma Admission (LINAA) group. This finding is clinically plausible given that IL-6 has been associated with increased risk of AAs[260], is known to be upregulated during exacerbations[669,670], and can be suppressed by both inhaled[671,672] and systemic steroids[673]. However, the clinical significance of this reduction remains unclear. While IL-6 is often linked to immune responses to viral infections[674], and has been proposed as a marker of airway epithelial activation[675], it is known to be upregulated by β2 agonists[676,677]. Given the high β2 agonist use expected during periods of symptom worsening and hospital treatment for acute asthma, it is difficult to determine how much of the IL-6 increase observed during AAs reflects true airway inflammation. Hence, the significance of the IL-6 reduction observed at V2 remains unclear.

A significant number of patients with increased, and even normal, markers of T2-high inflammation at V1 had elevated markers of T2-high inflammation at V2 despite systemic steroid treatment. This persistent inflammation may explain the association between a recent AA and the risk for a future one[678], as elevated T2-high inflammation is a well-recognised risk factor for AAs[389]. Whether this persistent T2-high inflammation reflects insufficient anti-inflammatory treatment is difficult to determine; however, it is in keeping with previous literature demonstrating ongoing T2-high activity despite systemic steroids treatment[583,594]. These observations highlight a subgroup of patients plausibly at higher risk of future attacks and challenge the treatment of asthma episodes irrespective of the underlying acute pathophysiology with little emphasis on follow-up and repeat measures of airway inflammation to assess treatment response.

Lung function and symptoms at recovery based on underlying inflammation at V1

Our study identified that improvement in lung function was significantly greater in those with T2-high compared to T2-low AAs. While this is biologically plausible given

the long-recognised relationship between T2-high inflammation and steroid responsiveness[168,445,447] and that benefits of steroids are limited to those with T2-high driven asthma[38,679], comparing the response to OCS between T2-high and T2-low phenotypes in the context of acute asthma has recently been investigated in the aforementioned PRISMA study. Couillard et al.[475] found a significant trend towards greater FEV_1% improvement in patients with T2-high/high (BEC ≥0.15 $x10^9$/L and FeNO ≥25 ppb). While a direct comparison with these results was not feasible as many of the patients in the present study had already commenced OCS at study recruitment, findings from the present study broadly support those of PRISMA despite some differences in the methodological designs between the studies. Collectively, findings from the present study and those from PRISMA extend the well-known relationship between T2-high asthma and steroid responsiveness to the settings of acute asthma and challenge the current practice of treating acute episodes of asthma uniformly.

Furthermore, also in agreement with the findings of the PRISMA study, the present study did not find differences in symptom score improvement between T2-high and T2-low AAs. This implies that symptom burden is equally high in both phenotypes. Indeed, symptom worsening is an unreliable indicator of the underlying disease activity during AAs. McDowell et al.[457] phenotyped exacerbations in adults with severe asthma and reported similar increases in symptoms in both the T2-high and T2-low groups. Similar findings have also been reported by the MEX study[455]. Collectively, these findings highlight the limitations of traditional symptom-based management algorithms and underscore the importance of considering the underlying disease process when assessing and treating AAs.

Utility of FeNO in predicting likelihood OCS responsiveness

We have previously demonstrated that FeNO correlates with and predicts sputum eosinophilia (Chapter 3). However, the utility of FeNO in predicting the likelihood of OCS responsiveness remained an open question until follow-up data became available. The present study demonstrated that a FeNO level of 24 ppb at V1 had a high specificity in identifying patients who achieved a minimum clinically important FEV_1% improvement. While this is logically expected given the previously recognised correlation between FeNO and sputum eosinophils[455,589-591,594] and that improvement

in FEV_1% following OCS treatment for acute asthma correlates with reduction in sputum eosinophils[447,583], this remains a novel and interesting finding that warrants formal validation. Although, as discussed in Chapter 3 and observed in findings from the PRISMA[475] study, BEC and FeNO are likely to act synergistically in predicting treatment response, results from the present study strongly support efforts towards a treatable trait and a precision medicine approach of acute asthma management recently demonstrated in both the PRISMA[475] and the ABRA studies[430].

Study strengths and limitations

The main strength of the present study is its broad inclusion criteria. Unlike previous studies, including PRISMA, which focused on patients with objectively verified asthma, the present study captured a more diverse and heterogeneous real-world population. Therefore, this study reflects the typical clinical scenarios encountered in acute care settings and specialist centres, where not all patients presenting with acute symptom deterioration suggestive of asthma have a confirmed prior diagnosis or airflow limitation suggestive of acute asthma. Further, the demographics of patients who attended the follow-up visit are largely comparable to those of patients at V1, suggesting that population bias is unlikely. Moreover, although the drop-out rate was relatively high, all patients in the subgroup analyses (LAARA and LINAA) completed V2 and the vast majority completed study assessment at both visits. Furthermore, given that lung function typically stabilises within 14 days following systemic steroid treatment[155,156], we believe the findings from V2 represent true recovery following hospitalisation and OCS treatment.

Limitations to the present study include the high dropout rate, as the inclusion of these patients may have influenced the overall findings. Further, the number of patients in the LINAA group is relatively small though sufficient to perform paired statistical analyses. In addition, we aimed to assess recovery in the NARA group, who may represent those admitted with asthma-mimicking conditions; however, only 3 patients of this group attended V2, but the study team believes the LINAA likely represents a mixture of patients with poorly controlled asthma and some with asthma mimicking conditions. Further, it is possible that some patients had an impending relapse at the time of the follow-up visit, which can occur within the 4 weeks following discharge[680].

However, although the present study was not designed to assess relapse, this is unlikely given the significant lung function and symptom improvement in those with airflow limitation at V1 (LAARA).

Conclusion

Our study provides a detailed characterisation of acute asthma episodes both during hospitalisation and recovery. A significant proportion of patients admitted and treated for suspected asthma had no objective evidence of airflow limitation suggestive of acute asthma. The degree of recovery following hospitalisation and treatment for AAs demonstrated significant variation depending on the underlying acute inflammatory phenotype. Importantly, point-of-care measurement of inflammatory markers has been demonstrated feasible during symptom worsening and have value in predicting treatment response, highlighting the importance of considering the inflammatory process underlying AAs.

These findings highlight the limitations of the current management guidelines largely based on non-asthma-specific symptoms with little emphasis on objective confirmation of lung function deterioration or consideration of the underlying inflammatory phenotype.

Chapter 6: Summary of findings, discussion, and suggestions for future research

Summary of findings

Results from the retrospective audit of 311 asthma attack admissions indicate a significant proportion of such admissions do not have documented evidence supportive of airflow obstruction suggestive of acute asthma. A small number of repeatedly admitted patients were more likely to have admissions with no obvious obstruction. These patients were typically on higher treatment steps, had a higher prevalence of asthma-mimicking conditions, and frequently required asthma-related ITU admission. Interestingly, the odds of having a no obvious obstruction admission increased progressively with the number of admissions per patient. The demographic features and medical history of those with repeat admissions are consistent with previous descriptions of frequent asthma exacerbators, patients with asthma mimics, and those with asthma attack misdiagnosis.

Our longitudinal, prospective clinical study of 100 asthma attack admissions at Nottingham University Hospitals further confirms findings from the audit, demonstrating that about 23% of the patients with objectively assessed lung function did not have evidence of airflow limitation suggestive of acute asthma. Although demographic and clinical characteristics were not significantly different between patients with and without airflow obstruction, comparison between those with airflow obstruction and those with non-asthma-related admissions (both normal lung function and inflammatory markers) demonstrated important distinctions that support the audit findings. Those with non-asthma-related admissions were on higher asthma treatment steps and had more frequent asthma-related healthcare utilisation, including GP and A&E visits, OCS bursts, and even hospitalisations compared to those with evidence of airflow obstruction, consistent with findings from the audit and previous descriptions of patients with common asthma mimics. Notably, all patients without objective evidence of airflow obstruction had a prior diagnosis of asthma, suggesting a possible association between a pre-existing asthma diagnosis and the likelihood of receiving an asthma attack diagnosis during subsequent episodes of asthma-like symptoms.

A discrepancy in airflow obstruction assessed using spirometry and FOT was noted, likely explained by the involvement of the small airways detected by FOT but not spirometry, as measures of the small airways were noted to be decreased at hospital admission but significantly improved at follow-up visits. POC measures of airflow obstruction and inflammation were well tolerated, even in very unwell patients. Importantly, POC FeNO measurement showed utility as a marker of T2-high inflammation, demonstrating a moderate correlation with sputum eosinophil counts and reliably predicting the well-established 3% sputum eosinophil threshold signifying eosinophilic airway inflammation, even following initiation of OCS.

Longitudinal follow-up analysis demonstrated that patients without objective evidence of acute asthma at hospital admission had largely unchanged lung function but significantly improved symptom scores at V2, suggesting that symptoms worsening at presentation may have been due to non-acute asthma or non-asthma related causes. A substantial number of patients had persistent T2-high inflammation at V2 despite OCS treatment, indicating a potentially high-risk group for future exacerbations. Interestingly, the response to OCS treatment varied depending on the inflammatory phenotype of attack. Patients with a T2-high attack phenotype had a significantly greater lung function improvement compared to those with T2-low attacks, further extending the long-recognised relationship between T2-high inflammation and steroid responsiveness to the context of acute asthma. Symptom improvement did not differ significantly between the T2-high and T2-low groups, highlighting the flaws of the largely symptoms-oriented treatment guidelines with little consideration of underlying disease pathophysiology. Notably, FeNO measurement at V1 demonstrated high specificity in predicting minimal clinically important improvement in lung function, suggesting inflammatory phenotyping at the time of attack may help guide acute asthma management, however this requires further validation.

Findings from both the retrospective audit and the prospective clinical study highlighted the complexity and heterogeneity of events currently labelled as asthma attacks. A substantial proportion of admissions lacked objective evidence of airflow obstruction, challenging conventional diagnostic paradigms and highlighting the potential for misdiagnosis. These observations, combined with varied responses to systemic corticosteroids, point towards significant gaps in the current understanding

and management of acute asthma. The following section will discuss the implications of these findings, the limitations of the the-one-size-fits-all approach, and highlight the potential of biomarker-driven, "treatable-traits" based strategies to refine the diagnosis and personalise the management of acute asthma episodes.

Discussion

Objective confirmation of lung function deterioration

Airway inflammation and airflow limitation are key components of the acute asthma disease process. While the inflammatory mechanisms remain incompletely understood, airflow limitation is generally straightforward to assess and confirm, with well-documented distinctions between episodes of poorly controlled asthma and true asthma attacks[155,156]. However, objective confirmation of airflow obstruction during acute asthma episodes is frequently overlooked[565,566], and when assessed, PEFR is not always reliable. Consequently, the literature includes numerous cases where patients are initially treated for acute asthma but are later found to have non-asthma-related causes for their presentation[433,526,569,570], and the present thesis provides a systematic evaluation of this important issue.

The most significant implication of this issue is the unnecessary treatment with OCS, which is far from inconsequential. OCS-associated morbidity is well-documented[206,207,681,682], with evidence indicating that even a single short course can result in adverse effects[206]. In addition, escalation of asthma treatment and access to asthma biologic treatment is often based on the count of OCS bursts prescribed for subjectively defined asthma attacks[683-685] under the assumption that these episodes are steroid and hence biologic responsive. Importantly, McDowell et al.[686] assessed OCS-associated toxicities in a cohort of biologics-eligible patients with severe asthma and found that all the 101 patients had OCS-related morbidities; these morbidities were significantly correlated with both the cumulative prednisolone dose and the number of exacerbations in the preceding year.

These findings highlight a critical limitation in the current approach to defining asthma attacks, which often relies on non-specific symptoms with little emphasis on objective confirmation of airflow limitation. This may, in part, account for previously reported

rates of asthma attack misdiagnosis[493,494] and potentially overestimation of asthma-related mortality rates[190,687-689]. Objective assessment and confirmation of airflow limitation is key if the evaluation and management of acute asthma episodes is to improve. A common misconception is that acutely obstructed patients are unable to perform reliable spirometry; however, Gostavo J.[690] refutes this assumption, stating, "there are no excuses for not performing spirometry in acute asthmatics in the emergency department setting" in his commentary on the study by Silverman et al.[502], which demonstrated that the majority of acutely obstructed asthma patients were able to perform acceptable and reproducible spirometry manoeuvres within the first hour of emergency department presentation. The aforementioned study also demonstrated that staff without prior spirometry experience could be rapidly trained to achieve acceptable performance standards. It is therefore imperative that spirometry, as well as FOT, given that both measures reflect different aspects of the lung mechanics, are incorporated into the assessment of patients presenting with symptoms suggestive of acute asthma. Although this may involve an initial financial investment, it is unlikely to exceed the long-term and substantial healthcare costs associated with both acute and chronic OCS-related adverse effects estimated at $512 per patient per year[691] (adjusted to 2025 US dollar), including acute myocardial infarction ($37,635 patient/year), bone fractures ($26,099 patient/year), and peptic ulcer ($31,028 patient/year)[207].

The one-size-fits-all approach

The understanding of the pathobiology of chronic asthma has substantially evolved over the years, enabling the development of precise, treatable-trait-based management algorithms guided by biomarkers of T2 inflammation that predict both exacerbation risk and treatment response[692]. In contrast, asthma attacks remain poorly understood and continue to be uniformly treated with OCS based on knowledge dating back to the 1950s[167] despite the limited data describing treatment response, its potential, or *likely*[550], variable efficacy[512-514], well-documented toxicity[686] and substantial treatment failure rate[693,694].

The widespread use of OCS for all episodes of asthma symptoms worsening is, in part, a consequence of the lack of a clear and objective definition of these events coupled with a lack of alternative treatment options, but largely driven by the longstanding

assumption that asthma is a uniformly steroid-responsive, eosinophilic disease, an assumption that has proven to be largely inaccurate in both biologic-treated[455] and non-biologics[457] settings. The pathophysiological mechanisms underlying chronic asthma cannot be presumed to apply to acute exacerbations, and it has been suggested that asthma control and exacerbations may be driven by distinct underlying disease mechanisms[179]. Indeed, evidence suggests that episodes of poor asthma control often respond well to short-acting β2 agonists, whereas exacerbations requiring hospitalisation and OCS treatment tend to be less responsive[156], and that even those on high dose ICS[695,696] and biologics[455] still experience exacerbations. Better understanding of the acute pathophysiology of asthma and associated inflammatory mechanisms is likely to improve acute asthma management and outcomes.

The reliance on relapse rates[697] and the need for hospital admission[698] to assess the efficacy of OCS in acute asthma without consideration of the underlying pathophysiological mechanisms is problematic and reflects a limited understanding of the acute disease process. In addition, many of the randomised controlled trials evaluating OCS efficacy were conducted before the widespread use of ICSs, which are known to reduce T2-high inflammation and improve both symptoms and lung function[699,700]. Thus, the efficacy reported in these earlier trials may be overstated, potentially reflecting steroid-responsive, T2-high events that could have been prevented, or even managed, with appropriate ICS use. Accurate phenotyping of these events, similar to that observed in studies of anti-T2 biologics demonstrating greater efficacy in those with elevated markers of T2-high inflammation[701,702], could have significantly altered the interpretation of these trials; however, these studies were conducted at a time when inflammatory biomarkers were not widely accessible.

The strongest evidence of varying response to OCS treatment in asthma attacks comes from the recently published PRISMA study by Couillard et al.[475], which demonstrated varying responses to OCS based on inflammatory profiles despite similar rates of adverse events across the groups. Findings from this work and from PRISMA not only highlight the utility of POC measurement of inflammation but also support previous calls for more selective OCS prescription[145], underscore the heterogeneity of AAs, and demonstrate that biomarker-directed management may be important in guiding treatment decisions in AAs, especially since stable asthma and the previous

exacerbation phenotype may not be linked to future exacerbation phenotype[455,457,461]. As there may be no difference in lung function decline or symptomatology between T2-high and T2-low AAs[455,457,639], inflammatory phenotyping would be a key facet of any such future biomarker directed approach.

The treatable traits

The recognition of the complexity and heterogeneity of the aetiology, disease mechanism, and manifestations of stable asthma has led to the abandonment of the one-size-fits-all approach and the development of personalised, treatable-traits -based management strategies. On the other hand, AAs continue to be classified following the 19th century diagnostic label principles described by Sir William Osler, linking disease manifestations (signs and symptoms) to anatomical and histopathological findings[148], which has been argued to be outdated, lead to suboptimal disease management, impedes the development of personalised treatment strategies[703], and, as argued by Ramsahai et al.[425], "is likely to only take us so far." Indeed, the classical Oslerian paradigm over-generalises patho-phenotypes and likely fails to capture the diversity of endotypes and phenotypes underlying acute asthma pathology.

Pavord et al.[145] argues that, as the inflammatory phenotype during acute asthma can often be foreseen from the stable state, the treatable trait is "just as applicable in patients presenting during an acute attack." While this is generally true, McDowell et al.[704] estimate, based on findings from two large exacerbation phenotyping studies, that approximately 75% of attack phenotypes mirror the stable disease phenotypes. Findings from the MEX study demonstrated heterogenous inflammatory profiles within the T2-high compartment, where patients with a stable T2-high asthma may still experience T2-high exacerbations driven by an ongoing T2-high mechanisms distinct from those underlying their stable disease. As such, although targeting pre-existing inflammation might be useful, it is likely that precise phenotyping and targeting of the acute pathology is the way forward to allow a more personalised approach of managing these episodes, perhaps allowing the identification of those likely to respond to OCS, or, more ambitiously, ending the era of OCS and targeting the specific acute pathology using available monoclonal antibodies guided by existing theranostic biomarkers[705].

The most notable application of the treatable traits approach in acute asthma to date is the recent study by Bafadhel et al.[430], which targeted eosinophilic exacerbations (based on blood eosinophils) irrespective of diagnostic label. The study demonstrated that treatment with Benralizumab resulted in fewer treatment failures and adverse events, along with greater improvement in symptoms and quality of life, compared to standard care treatment with prednisolone.

FeNO is also a well-established marker of T2-high inflammation and is likely to be an important treatable trait in acute asthma. Elevated FeNO is associated with poor asthma control[590,706,707] and is frequently observed during asthma attacks[584,605,606]. The potential for a FeNO-targeted treatment approach was highlighted by findings from the MEX study[708], which offered a unique insight into episodes of asthma worsening characterised by isolated FeNO increase in patients receiving anti-IL-5 therapy. These episodes were often clinically mild, potentially reflecting poorly controlled asthma rather than true exacerbations. Given that FeNO is also a reliable biomarker of response to anti-IL-4/IL-13 therapies[709], isolated FeNO increase may represent a distinct, targetable, and clinically relevant trait of asthma worsening, however this hypothesis requires further validation. The degree of clinical recovery and inflammatory response to OCS in FeNO-high versus FeNO-low attacks in patients on anti-IL4Rα, anti-IL5, and anti-IL5Rα is currently being investigated in the BOOST study by Pavord et al[710].

The role of the small airways in the pathophysiology of asthma has historically been under-recognised. However, studies have consistently shown that small airway dysfunction is highly prevalent[97], and that the lung periphery is involved in many of the mechanisms of asthma including inflammation[115,116], AHR[125], airway remodelling,[113] symptomatology[102], and lung function decline[122,125]. SAD has also been implicated in poor asthma outcomes, including impaired disease control[101,102] and increased exacerbation risk[104,109,110]. The role of SAD in the clinical expression of AAs, however, is less well understood, but studies, including ours, indicate that the small airways are impaired during acute asthma[326,475,619] and tend to improve during recovery[475,619], implying a potential role of SAD in the pathophysiology of acute asthma.

Small airway dysfunction may be an important trait during exacerbations as it fits the criteria of a treatable trait, that is, relevant, measurable, and, potentially, treatable. Indeed, although trials evaluating the efficacy of ICS in the treatment of acute episodes

of asthma has been conflicting[372], perhaps due to the limited peripheral deposition of conventional ICS, some trials have shown a superior efficacy of ICS in reducing the need for hospitalisation and improving clinical outcomes compared to a placebo[157], and even systemic steroids[711].

Moreover, scintigraphic studies[712] demonstrate that drug deposition in the lung periphery is higher with fine particles, and lead to greater reduction of ICS dosage, improved asthma control and improved quality of life. In addition, fine particle ICS have demonstrated greater efficacy in controlling symptoms and reducing the risk of AAs compared to routinely available ICS-containing inhalers with standard particle size[106-108]; however, this has not been evaluated during exacerbations. Notably, the clinical advantages of fine-particle ICS were observed despite comparative studies using FEV_1 (known to reflect the large airway function) as an endpoint, and without selection of patients with confirmed SAD, in whom even greater benefit might be expected. This raises the question of whether precise targeting of the small airways in patients with documented SAD could reduce the need for OCS, and enable a more personalised, treatable-trait approach to managing acute asthma. However, as previously mentioned, SAD remains a poorly understood area, its diagnosis is inherently challenging[98], and the optimal particle size for targeting the distal airways has not been clearly established[713].

The absence of airflow obstruction may indicate either a period of poor asthma control during which lung function is likely preserved and treatment with SCS is likely unnecessary, or the presence of an extrapulmonary trait mimicking acute asthma for which steroid responsiveness is typically diminished. In cases of poorly controlled asthma, SCS might not be required, as evidence suggests that ICS-LABA is effective in reducing exacerbation rates in those with poor asthma control[158]. When an extrapulmonary trait is suspected, as indicated by the absence of airflow obstruction and normal inflammatory markers, treatment might involve input from other specialties. The avoidance of unnecessary SCS exposure in the absence of airflow obstruction is a key advantage of the treatable traits approach, as the final goal of the precision medicine is to "improve clinical outcomes for individual patients while minimizing unnecessary side effects for those less likely to respond to a given treatment[427]."

Suggestions for care in practice

Based on results from the present thesis, several practical recommendations can be proposed to guide more precise and effective care.

Objective confirmation of airflow limitation should be a standard component of the assessment of acute asthma. While FOT remains primarily a research tool requiring further validation before its integration into routine clinical practice, spirometry is a well-established, widely available tool and should be incorporated into emergency and inpatient protocols for evaluating patients presenting with symptoms suggestive of acute asthma deterioration. As this thesis and previous work demonstrated, spirometry is feasible and well-tolerated, even in acutely unwell patients. This would provide important information to distinguish true exacerbations from events of poorly controlled asthma and those of conditions mimicking acute asthma presentation. This consideration of the underlying lung physiology would enable a more accurate diagnosis, prevent unnecessary OCS use, and promote targeted care.

Our findings confirm the feasibility and clinical utility of POC measurement of T2-high inflammatory markers as predictors of response to systemic corticosteroids. However, the role of FeNO is in acute asthma is not-well understood and cannot currently be recommended for routine use in acute asthma management. In contrast, blood eosinophil count is a robust and well-established biomarker of T2-high activity and may be utilised to guide the management of acute asthma, provided that eosinophil levels are not suppressed by pre-admission corticosteroid use and that alternative diagnoses or contributing factors are carefully considered. We therefore recommend consideration of blood eosinophils at initial presentation to stratify patients based on likely steroid responsiveness. This approach would support more rational, biomarker-guided use of OCS and facilitate the avoidance of unnecessary corticosteroid exposure.

The conventional emergency and pulmonary medicine-led approach to managing episodes suggestive of acute asthma may be insufficient. An integrated, multidisciplinary strategy is likely essential to advance the management of acute asthma and improve both disease burden and patient outcomes. Findings from this thesis highlight that, in those with no evidence of acute asthma pathology, based on

the absence of objectively assessed lung function deterioration or elevated T2-high markers, a multidisciplinary management incorporating relevant specialities should be the standard of care when addressing presentations suggestive of acute asthma. This is particularly important for repeatedly admitted patients, as this group is at high risk for inappropriate treatment escalation and long-term harm. Information from acute episodes from this high burden group of patients can help identify whether extrapulmonary traits are contributing to symptom worsening and hospitalisations. This can guide more targeted investigations and management, potentially allowing for de-escalation of asthma treatment and prevention of further admissions through involvement of other specialties.

Suggestions for future research

Objectively verified lung function deterioration

As we have demonstrated that POC assessments of airflow obstruction, including spirometry and FOT, are both feasible and well tolerated even in acutely unwell patients, it would be of interest to compare the clinical outcomes, diagnostic accuracy, and treatment implications based on objective lung function assessments versus conventional, symptom-based management algorithms. Aaron et al.[500] previously demonstrated that 30% of 613 patients with a physician-diagnosed asthma do not have objective evidence of asthma, of whom 6% had alternative diagnoses known to mimic asthma. Worsening of asthma-like symptoms in those with asthma mimicking conditions could easily be misclassified as asthma attacks in the absence of objective confirmation. Observational longitudinal studies comprehensively investigating those with asthma-like clinical presentation but not objectively assessed lung function deterioration, followed by a randomised controlled trial comparing current management protocols to those based on objective airflow assessment, would help delineate long-term clinical course, treatment response, healthcare utilisation, and the contribution of extrapulmonary traits, ultimately enabling more accurate differentiation between true asthma attacks and mimicking conditions.

Precise targeting of acute asthma episodes

Following objective confirmation of lung function deterioration, inflammatory phenotyping of acute episodes is key to guide personalised treatment approaches and facilitate the avoidance of mass OCS prescription; however, such an approach is likely hindered by the limited understanding of the acute inflammatory mechanisms. Emerging studies such as PRISMA[475], MEX[618], and BOOST[710] are beginning to enrich our understanding of inflammatory events underlying acute asthma. Further observational studies, particularly targeting phenotypic clusters characterised by isolated FeNO increase and blood eosinophilia, are required to delineate the specific roles of these biomarkers. This may allow future interventional trials assessing whether treatment decisions based on T2-marker status improve outcomes compared to traditional steroid-based management, allowing a smooth transition away from the current OCS for all era towards the treatable traits and biologics era.

Further, as demonstrated by numerous studies, T2-low asthma attacks are commonly infection-driven[455,457] and associated with neutrophilic inflammation, a phenotype typically less responsive to corticosteroids, as opposed to T2-high events which typically respond well to OCS. However, both T2-low and T2-high asthma attacks are often managed similarly, with many patients receiving antibiotics irrespective of the underlying inflammatory profile[457,475]. Findings from the MEX[455] study demonstrated that exacerbations characterised by low FeNO (≤25 ppb) in patients treated with Mepolizumab were more likely to be pathogen-driven and treated with antibiotics, a novel demonstration of the potential utility of attack inflammatory profiling in guiding treatment decisions. The generalisability of these findings remains to be formally validated in placebo-controlled studies within the broader asthma population. Such a FeNO-oriented management strategy could support a more precise, treatable-traits-based approach to acute asthma care, enabling targeted use of antibiotics (particularly Azithromycin which has also demonstrated anti-viral and anti-inflammatory properties) in those with likely infection-driven, T2-low exacerbations and avoiding unnecessary corticosteroid exposure in those unlikely to benefit.

The obesity phenotype

The interaction between obesity and asthma symptomatology requires further investigation. Obesity and asthma appear to be linked[646-648], and obese individuals are more likely to receive an asthma diagnosis[649], often lacking an objective diagnostic confirmation[650,651], compared to non-obese. Obese patients are typically characterised by a T2-low inflammatory phenotype[714] and often report a higher burden of asthma-like symptoms[650,714,715] despite high intensity inhaled[6,714] and even systemic corticosteroids[714,715]. As demonstrated in chapter 4 and prior research[493], asthma attacks in obese individuals often lack objective evidence of airflow limitation. As this group is prone to frequent exacerbations[6,715], they represent a significant unmet clinical need. The pathophysiology of acute asthma-like episodes in this group remains poorly understood. A detailed consideration of pulmonary and extra-pulmonary traits underlying episodes of symptoms worsening, perhaps in a cross-sectional prospective, observational analysis of presumed attacks in obese compared to non-obese patients is likely to allow a thorough explanation of symptoms worsening of this important group. This would allow a deeper understanding of this phenotype and improving diagnostic precision and therapeutic strategies for this high-burden subgroup.

Concluding remarks

Clinical presentations typical of acute asthma may arise from a range of underlying causes; however, distinguishing true asthma exacerbations from asthma mimics is inherently challenging without objective assessment of lung physiology. The inflammatory mechanisms underlying true asthma attacks remain poorly understood, are likely heterogeneous, and cannot be reliably predicted from the stable state phenotype or previous exacerbation profiles. Current management guidelines place limited emphasis on objective confirmation of acute lung function deterioration, and the inflammatory mechanisms underlying attacks are often not considered, even though asthma mimics are common, and the benefits of steroids are often limited to eosinophilic inflammation. Objective assessment of airflow limitation is evidently feasible, supported by the availability of point-of-care tools such as spirometry and forced oscillation technique. However, inflammatory mechanisms during asthma attacks will likely remain an unmet need, pending more comprehensive investigation

during both the acute phase and recovery. Ultimately, the goal is to improve the recognition, description, targeted treatment and prevention of these critically important events to improve patient outcomes from asthma attacks and reduce unnecessary treatment and related side effects.

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Appendices

Appendix A: Audit of asthma attack admissions-summary of missing data.

Table 75. Demographic characteristics according to admission frequency-missing data.

Demographics	All patients (N= 220)	STA (n= 182)	MTA (n= 38)	FTA (n= 7)
	N (%)			
Age	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Gender	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Ethnicity	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Townsend score	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Number of admissions	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Length of stay	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Table 76. Medical history and comorbidities according to admission frequency-missing data.

Medical history and comorbidities	All patients (N= 220)	STA (n= 182)	MTA (n= 38)	FTA (n= 7)
	N (%)			
Previously diagnosed asthma	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Source of diagnosis	7 (3.2)	6 (3.3)	1 (2.6)	0 (0%)
Significant comorbidities	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Airway diseases	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Asthma Mimics	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Smoking status	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Asthma related ITU admission	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Table 77. Previously documented asthma history according to admission frequency-missing data.

Asthma-specific history	All patients (N= 220)	STA (n= 182)	MTA (n= 38)	FTA (n= 7)
	N (%)			
Asthma severity	36 (16.3)	31 (17.0)	5 (13.2)	2 (28.6)
Asthma phenotype	37 (16.8)	26 (14.3)	11 (28.9)	5 (71.4)
Objective evidence of asthma	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Attended asthma appointments (last 2 years)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Asthma appointments missed (last 2 years)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Table 78. Asthma treatment according to admission frequency-missing data.

Asthma treatment	All patients (N= 220)	STA (n= 182)	MTA (n= 38)	FTA (n= 7)
	N (%)			
On GINA treatment Step	0 (0%)	0 (0%)	0 (0%)	0 (0%)
On Biological treatment	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Table 79. Documented breath sounds and PEFR-missing data.

Objective evidence of airflow obstruction	All admissions (N=311)	AAs with EAO (n=260)	AAs with NOO (n=51)
	N (%)		
Breath sound	0 (0%)	0 (0%)	0 (0%)
PEFR	49 (15.8)	28 (10.7)	21 (41.2)

Table 80. Clinical characteristics in admission with vs. without evidence of airflow obstruction-missing data.

Clinical characteristics	All admissions (N= 311)	AAs with EAO (n=260)	AAs with NOO (n=51)
	N (%)		
On admission PEFR	49 (15.8)	28 (10.7)	21 (41.2)
Length of hospital stay	0 (0%)	0 (0%)	0 (0%)
Requiring ITU admission	0 (0%)	0 (0%)	0 (0%)
Acute asthma severity	0 (0%)	0 (0%)	0 (0%)
Abnormal CT/CXR findings	0 (0%)	0 (0%)	0 (0%)
Secondary diagnosis	0 (0%)	0 (0%)	0 (0%)
AA trigger identified	0 (0%)	0 (0%)	0 (0%)
Symptoms duration prior to admission	0 (0%)	0 (0%)	0 (0%)
Inflammation and infection			
BEC ≥0.4 x10^^{9}/L	0 (0%)	0 (0%)	0 (0%)
CRP ≥10 mg/L	0 (0%)	0 (0%)	0 (0%)
Microbiology results			
Positive Viral microbiology	160 (51.4)	131 (50.4)	29 (56.8)
Positive Bacterial microbiology	240 (77.2)	202 (77.7)	38 (74.5)

Table 81. Treatment received in admissions with vs. without evidence of airflow obstruction-missing data.

Treatment received	All admissions (N= 311)	AAs with EAO (n=260)	AAs with NOO (n=51)
	N (%)		
Pre-admission systemic anti-inflammatory	0 (0%)	0 (0%)	0 (0%)
Magnesium sulphate	0 (0%)	0 (0%)	0 (0%)
Theophylline	0 (0%)	0 (0%)	0 (0%)
Antibiotic treated	0 (0%)	0 (0%)	0 (0%)
SCS treated	0 (0%)	0 (0%)	0 (0%)
SCS duration in days	0 (0%)	0 (0%)	0 (0%)

Table 82. Discharge characteristics of admissions with vs. without evidence of airflow obstruction-missing data.

Discharge characteristics	All admissions (N= 311)	AAs with EAO (n=260)	AAs with NOO (n=51)
	N (%)		
On Discharge PEFR	116 (37.3)	79 (30.4)	37 (72.5)
PEFR >75% on discharge	116 (37.3)	79 (30.4)	37 (72.5)
PEFR% improvement	116 (37.3)	79 (30.4)	37 (72.5)
6 elements of good practice care	0 (0%)	0 (0%)	0 (0%)
Post admission Follow-up arranged	0 (0%)	0 (0%)	0 (0%)
• Post admission Follow-up attended	0 (0%)	0 (0%)	0 (0%)
• Post admission Follow-up missed	0 (0%)	0 (0%)	0 (0%)
Medication changes at discharge	0 (0%)	0 (0%)	0 (0%)

Appendix B: Audit of asthma attack admissions- Comparison between asthma attack admissions with and without evidence of airflow obstruction only considering first admission of each patient identified

Summary of findings

In total, 220 admissions, corresponding to the first admission of each of the 220 patients identified, were audited. 34 (15.5%) of the admissions did not have evidence of airflow obstruction (Table 83, Figure 39). LOS was lower, and the proportion of those with ITU admissions and secondary diagnoses were higher in the NOO admissions compared to admissions with EAO (Table 84). Similar to findings from the main admissions analysis, the proportion of those with increased BEC was lower in the NOO admissions (Table 84). Although median BEC was lower in those on pre-admission systemic anti-inflammatory treatment, the proportion of patients on pre-admission systemic anti-inflammatory was not different between the groups, and when only including patients not on systemic anti-inflammatory, the proportion of patients with increased BEC was still lower in the NOO admissions.

Variation in SCS treatment was similar to those observed in the main analysis; NOO admissions tended to be less likely to have received SCS treatment compared to admissions with EAO (Table 85), likely due to the involvement of secondary/contributing diagnoses.

On discharge median PEFR% and the proportion of patients with preserved PEFR% were higher in those with NOO (Table 86). The percentage of PEFR improvement was significantly higher in the admissions with EAO compared to those with NOO (Table 86).

Table 83. Documented breath sounds and PEFR.

Objective evidence of airflow obstruction	AA admissions (N=220)
	N (%)
Breath sound documented	220 (100%)
• *Wheeze+*	152 (69.1%)
• *No Wheeze*	68 (30.9%)
PEFR documented	194 (88.2%)
• *PEFR limitation (≤75%)*	151 (68.6%)
• *PEFR >75%*	43 (19.6%)
• *PEFR not performed/not documented*	26 (11.8%)

Figure 39. Evidence of airflow obstruction documented in admissions audited.

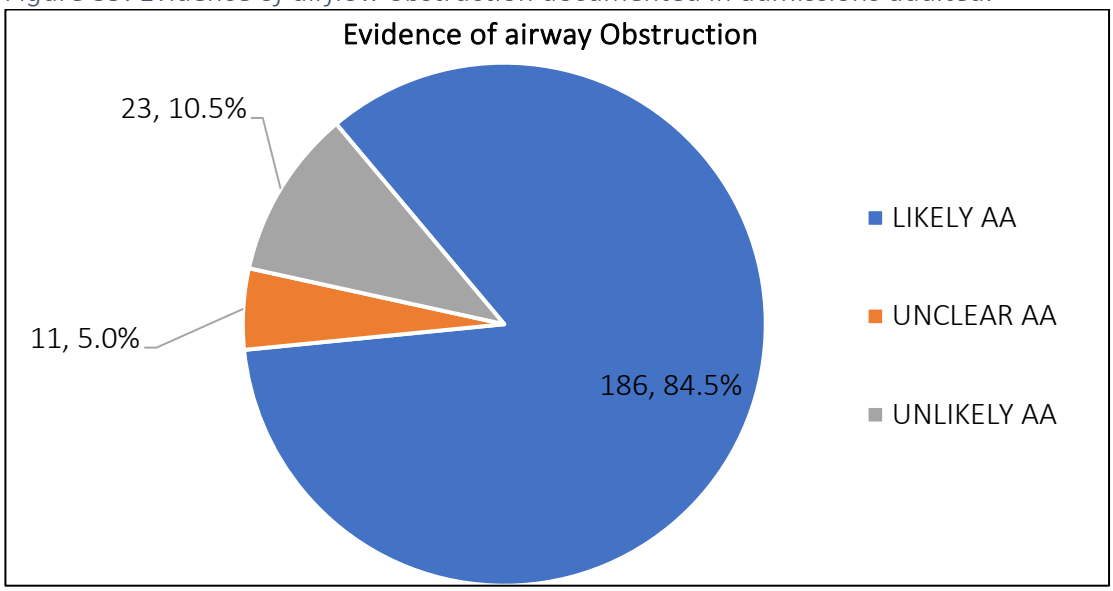


Table 84. Clinical characteristics in admission with vs. without evidence of airflow obstruction.

Clinical characteristics	All admissions (N= 220)	AAs with EAO (n=186)	AAs with NOO (n=34)	P value
	N (%) unless stated otherwise			
On admission PEFR Median (IQR) (n= 194)	52.0 (40.9 - 73.3)	50.0 (40.0 - 60.2)	81.8 (77.8 - 87.9)	<0.001
LOS Median (IQR)	3 (1 - 5)	3 (1 - 5)	2 (1 - 3)	0.07
Requiring ITU admission	3 (1.4)	1 (0.5)	2 (5.9)	0.06
Acute asthma severity*				
• *No signs of acute asthma*	26 (11.8)	11 (5.9)	15 (44.1)	<0.001
• *Moderate*	55 (25.0)	55 (29.6)	0 (0)	
• *Acute severe*	54 (24.6)	41 (22.0)	13 (38.2)	
• *Life-threatening*	58 (26.4)	54 (29.0)	4 (11.8)	
• *Near-fatal*	27 (12.3)	25 (13.4)	2 (5.9)	
Abnormal CT/CXR findings	12 (5.5)	10 (5.4)	2 (5.9)	1
Secondary diagnosis	98 (44.6)	76 (40.9)	22 (64.7)	0.01
• Exacerbation of other airway diseases**	6 (2.7)	3 (1.6)	3 (8.8)	0.05
• Pneumonia	14 (6.4)	12 (6.5)	2 (5.9)	1
• LRTI	65 (29.6)	50 (26.9)	15 (44.1)	0.04
• *ILO*	1 (0.5)	0 (0)	1 (2.9)	0.15
• BPD	2 (0.9)	1 (0.5)	1 (2.9)	0.28
• Anxiety/Depression	2 (0.9)	2 (1.1)	0 (0)	1
AA trigger identified	116 (52.7)	103 (55.4)	13 (38.2)	0.07
• Coryzal/flu-like symptoms	102 (46.4)	92 (49.5)	10 (29.4)	0.03
• Pollutants***	5 (2.3)	3 (1.6)	2 (5.9)	0.17
• Outdoor allergens****	5 (2.3)	5 (2.7)	0 (0)	1
• Cold air & changing weather	3 (1.4)	2 (1.1)	1 (2.9)	0.39
• Exercise	1 (0.4)	1 (0.5)	0 (0)	1
Symptoms duration prior to admission (in days) Median (IQR)	3.0 (1.0 - 7.0)	3.0 (1.0 - 7.0)	2.5 (1.3 - 9.3)	0.87
Inflammation and infection				
BEC ≥0.4 x10^9/L	51 (23.2)	47 (25.3)	4 (11.8)	0.09
CRP ≥10 mg/L	92 (41.8)	79 (42.5)	13 (38.2)	0.65
Microbiology results				
Positive Viral microbiology†	49 of 108 (45.4)	44 of 95 (46.3)	5 of 13 (38.5)	0.59
Positive Bacterial microbiology‡	1 of 55 (1.8)	1 of 47 (2.1)	0 of 8 (0)	0.85

**As per the BTS guideline. **COPD/bronchiectasis. ***Smoke, aerosol fresheners, cleaning products, perfumes. ****Pollen/trees. †In those with viral PCR results available. ‡In those with sputum Mc&s results available. LOS: Length of hospital stay. LRTI: Lower Respiratory Tract Infection. ILO: Inducible laryngeal obstruction. BPD: Breathing Pattern Disorders. BEC: Blood eosinophil count. CRP: C-reactive protein.*

Table 85. Treatment received in admissions with vs. without evidence of airflow obstruction.

Treatment received	All admissions (N= 220)	AAs with EAO (n=186)	AAs with NOO (n=34)	P value
	N (%) unless stated otherwise			
Pre-admission systemic anti-inflammatory*	34 (15.5)	29 (15.6)	5 (14.7)	0.89
Magnesium sulfate	26 (11.8)	22 (11.8)	4 (11.8)	1
Theophylline	10 (4.6)	9 (4.8)	1 (2.9)	1
Antibiotic treated	102 (46.4)	89 (47.9)	13 (38.2)	0.30
SCS treated	217 (98.6)	185 (99.5)	32 (94.1)	0.06
SCS duration in days (Mean ± SD) (n= 217)	7.4 ± 2.0	7.4 ± 1.9	7.4 ± 2.2	0.91

**Low-dose maintenance OCS, biologics, or pre-admission treatment with OCS. SCS: systemic corticosteroids.*

Table 86. Discharge characteristics of admissions with vs. without evidence of airflow obstruction.

Discharge characteristics	All admissions (N= 220)	AA with EAO (n=186)	AA with NOO (n=34)	P value
	N (%) unless stated otherwise			
On Discharge PEFR%* Median (IQR) (n= 148)	80.0 (66.8 - 92.4)	77.9 (65.3 - 89.5)	96.8 (85.3 - 103.6)	**<0.001**
PEFR >75% on discharge*	87 (58.8)	75 (55.2)	12 (100)	**0.001**
PEFR% improvement* (Mean ± SD)	33.2 ± 17.2	34.8 ± 16.9	14.8 ± 6.8	**<0.001**
6 elements of good practice care**	73 (33.2)	65 (34.9)	8 (23.5)	0.19
Post admission Follow-up arranged	**135 (61.4)**	**117 (62.9)**	**18 (52.9)**	0.27
• Post admission Follow-up attendees	110 (81.5)	94 (80.3)	16 (88.9)	0.52
• Post admission Follow-up missed	25 (18.5)	23 (19.7)	2 (11.1)	
Medication changes at discharge	81 (36.8)	71 (38.2)	10 (29.4)	0.33

**In admissions with PEFR documented on admission and on discharge. **As recommended by the National Asthma and COPD Audit Programme (NACAP).*

Appendix C: APEX3- study protocol

Asthma: Phenotyping Exacerbations 3

Final version 1.0
10TH November 2021

Short title:	**A**sthma: **P**henotyping **EX**acerbations 3
Acronym:	APEX 3
Trial Registration:	www.clinicaltrials.gov
ISRCTN:	Not Applicable
IRAS Project ID:	304615
Trial Sponsor:	University of Nottingham
Sponsor reference:	*21066*
Funding Source:	Internal Funds

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SYNOPSIS

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

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Eligibility criteria	Inclusion Criteria: • The ability to give fully informed consent • Male or female aged ≥ 18 years of age. • Admission with a suspected acute asthma attack. • Able (in the investigator's opinion) and willing to comply with clinical investigation requirements. Exclusion Criteria • Other clinically significant respiratory diseases including predominant Chronic Obstructive Pulmonary Disease (COPD) and bronchiectasis. • Any other clinically significant medical disease or uncontrolled concomitant disease that is likely, in the investigator's opinion, to impact the ability to participate in the study or the study results. • Pregnant women, lactating women or women who are planning to become pregnant. • Investigator determined apparent other cause for admission. • Acute COVID infection. • Non-English-speaking participants who are unable to comprehend the reasons for the study due to limitations in understanding the English language.
Description of interventions	• The collection of medical history, demographic data, and concomitant medications. • The incidence of upper airway symptoms will be assessed using the Sino-nasal Outcome Test (SNOT). • The assessment of the incidence of Gastroesophageal Reflux Disease using the GERDQ questionnaire. • The assessment of the incidence of vocal cord dysfunction using the Pittsburgh Vocal Cord Dysfunction (VCD) Index. • The assessment of the incidence of dysfunctional breathing using the Nijmegen dysfunctional questionnaire. • The assessment of the incidence of anxiety and depression using the Hospital Anxiety and Depression Scale (HADS). • The assessment of the quality of life using the Acute Asthma Quality of Life Questionnaire. • The assessment of medication adherence using the Medication Adherence Report Scale. • The assessment of lung function by performing Fractional Exhaled Nitric Oxide (FeNO), Forced Oscillation Technique (FOT), Peak Expiratory Flow Rate (PEFR) and Spirometry. • The collection of blood samples for laboratory analysis and storage for future research. • The collection of sputum samples for sputum differential cell count, bacterial culture and sensitivity and storage for future research. • The collection of nasal absorption samples for future cytokine profiling. • The collection of samples via nasal lavage and throat swabs for viral identification. • The collection of optional nasal brushing samples for RNA and DNA extraction.
Duration of study	Study duration: from May 2022 to March 2024.

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

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ABBREVIATIONS

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

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

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STUDY BACKGROUND INFORMATION AND RATIONALE

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

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

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

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

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

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

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STUDY OBJECTIVES AND PURPOSE

PURPOSE

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

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

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

PRIMARY OBJECTIVE

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

SECONDARY OBJECTIVES

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

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DETAILS OF PRODUCT(S)

Description of Products

Saline

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

Salbutamol

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

Packaging and labelling

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

Storage, dispensing and return

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

Placebo
No Placebo is being used.

Known Side Effects

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

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

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STUDY DESIGN

STUDY CONFIGURATION

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

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

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

Primary endpoint

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

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

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Safety endpoints

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

Stopping rules and discontinuation

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

RANDOMIZATION AND BLINDING

There is no randomisation in this cohort study.

STUDY MANAGEMENT

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

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

DURATION OF THE STUDY AND PARTICIPANT INVOLVEMENT

Study Duration:

We plan to recruit approximately 100 patients over a period of 22 months. The total duration of the study will be approximately 2.5 years. The study will begin in May 2022 and finish in March 2024.

Participant Duration:

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

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

End of the Trial

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

SELECTION AND WITHDRAWAL OF PARTICIPANTS

Recruitment

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

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

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

Eligibility criteria

Inclusion Criteria

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

Exclusion Criteria

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

Expected duration of participant participation

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

Removal of participants from therapy or assessments/Participant Withdrawal

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

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

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Informed consent

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

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

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

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

STUDY TREATMENT AND REGIMEN

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

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

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

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

Study Assessments Algorithm.

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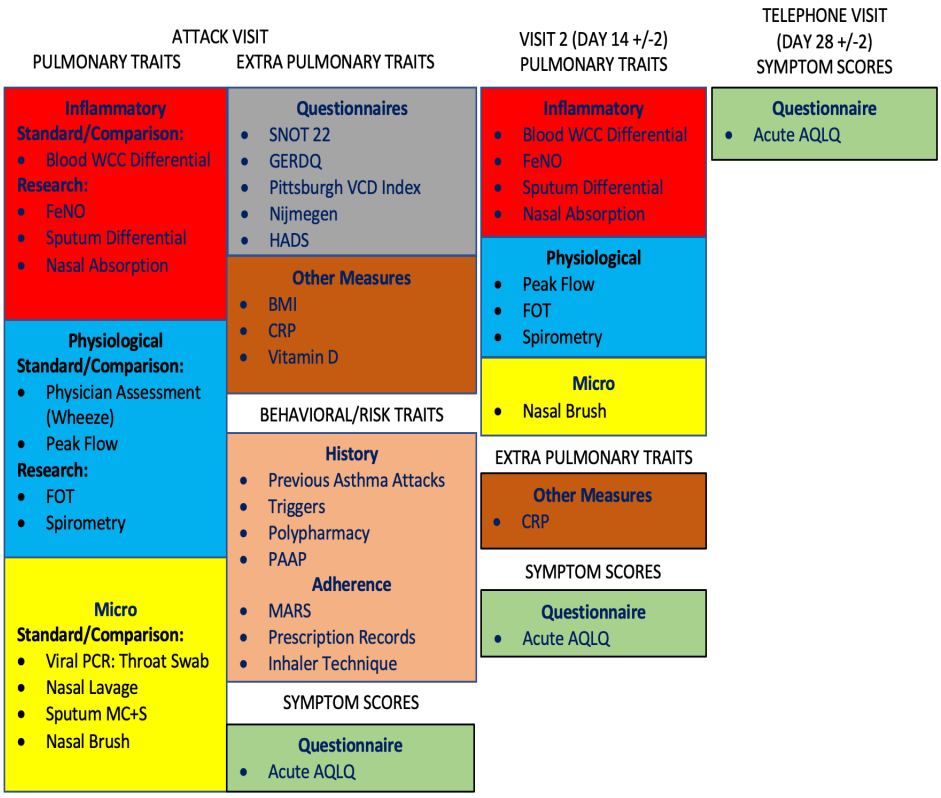


Figure 1: Study assessments algorithm.
Definition of abbreviations: WCC = white cell count; FeNO = fractional exhaled nitric oxide; FOT = forced oscillation technique; PCR = polymerase chain reaction; MC+S = microscopy culture + sensitivity; SNOT = Sino-nasal outcome test; GERDQ = gastroesophageal reflux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

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Schedule of study assessments.

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

Table 1: Schedule of study assessments.

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Study Assessments

Medical History, Demographics and Concomitant Medications

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

Height, Weight and Blood Pressure

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

Sino-nasal Outcome Test

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

Gastroesophageal Reflux Disease questionnaire.

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

Pittsburgh Vocal Cord Dysfunction Index

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

Nijmegen Questionnaire.

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

Hospital Anxiety and Depression Scale

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

Medication Adherence Report Scale

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

Acute Asthma Quality of Life Questionnaire

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

Fractional Exhaled Nitric Oxide (FeNO)

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

Forced Oscillation Technique (FOT)

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

Spirometry

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

Induced and Spontaneous Sputum

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

Laboratory Assessments

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

Blood Samples for Biomarkers

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

Nasal Absorption

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

Nasal Lavage

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

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

Nasal Epithelium Sample

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

Throat Swab

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

Compliance and adherence

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

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

Criteria for terminating trial

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

TRANSPORT AND STORAGE OF THE TISSUES

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

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

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

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

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

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

LABORATORY ANALYSES

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

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

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

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

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

Laboratory analyses will be detailed in a study laboratory manual.

STATISTICS

Methods

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

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

Sample size and justification

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

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Assessment of safety

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

Procedures for missing, unused and spurious data

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

ADVERSE EVENTS

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

Definitions

Adverse Events

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

An AE does include a / an:

1. An exacerbation of a pre-existing illness.

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

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

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

An AE does not include a / an:

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

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

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

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

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

Serious Adverse Events

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

1. Death

2. A life-threatening adverse event

3. Inpatient hospitalisation or prolongation of existing hospitalisation

4. A disability / incapacity

5. A congenital anomaly in the offspring of a participant

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

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

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

Causality

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

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

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

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

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

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Reporting of adverse events

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

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

Trial Investigation Related SAEs

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

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

The Chief Investigator will:

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

Participant removal from the study due to adverse events

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

ETHICAL AND REGULATORY ASPECTS

ETHICS COMMITTEE AND REGULATORY APPROVALS

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

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

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

INFORMED CONSENT AND PARTICIPANT INFORMATION

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

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

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

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

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

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

RECORDS

Case Report Forms

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

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

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

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

Sample Labelling

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

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

Source documents

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

Direct access to source data / documents

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

DATA PROTECTION

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

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

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

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QUALITY ASSURANCE & AUDIT

INSURANCE AND INDEMNITY

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

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

TRIAL CONDUCT

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

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

TRIAL DATA

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

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

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

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RECORD RETENTION AND ARCHIVING

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

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

DISCONTINUATION OF THE TRIAL BY THE SPONSOR

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

STATEMENT OF CONFIDENTIALITY

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

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

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

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

PUBLICATION AND DISSEMINATION POLICY

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

USER AND PUBLIC INVOLVEMENT

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

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

STUDY FINANCES

Funding source

Internal Funds

Participant stipends and payments

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

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SIGNATURE PAGES

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Date: 22/2/22

Co- investigator: (name) _____

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Date: _____

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Date: 22/2/22

Trial Statistician: (name)__Tricia McKeever_____

Signature:__  _____

Date: __23/02/22_____

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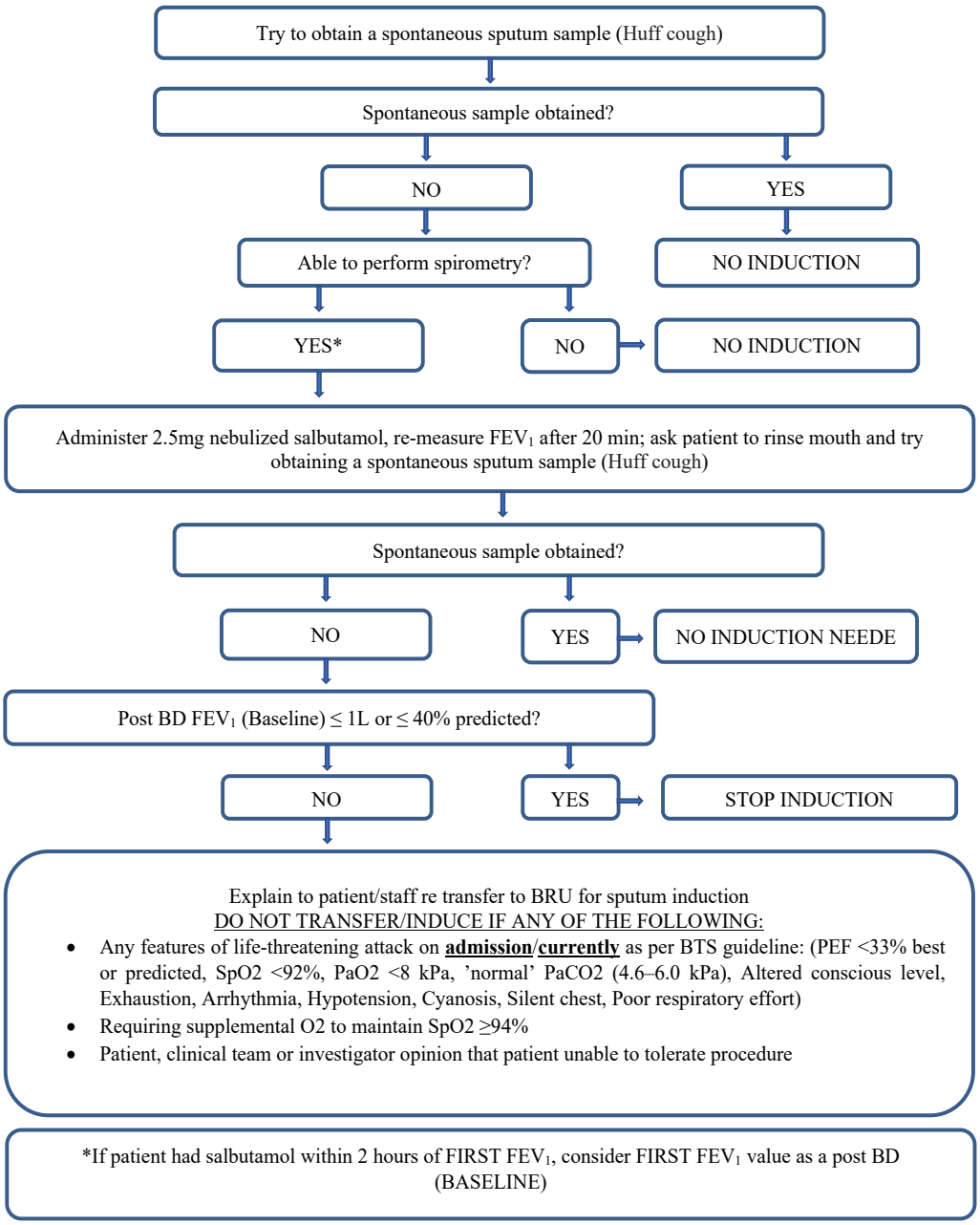
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Appendix D: APEX3- Sputum induction protocol

Nottingham Respiratory Research Unit
• Asthma • COPD • ILD • Lung Infection •

Nottingham University Hospitals NHS
NHS Trust

APEX3
Sputum induction protocol flowchart
Safety criteria



Explain to patient/staff re transfer to BRU for sputum induction
DO NOT TRANSFER/INDUCE IF ANY OF THE FOLLOWING:

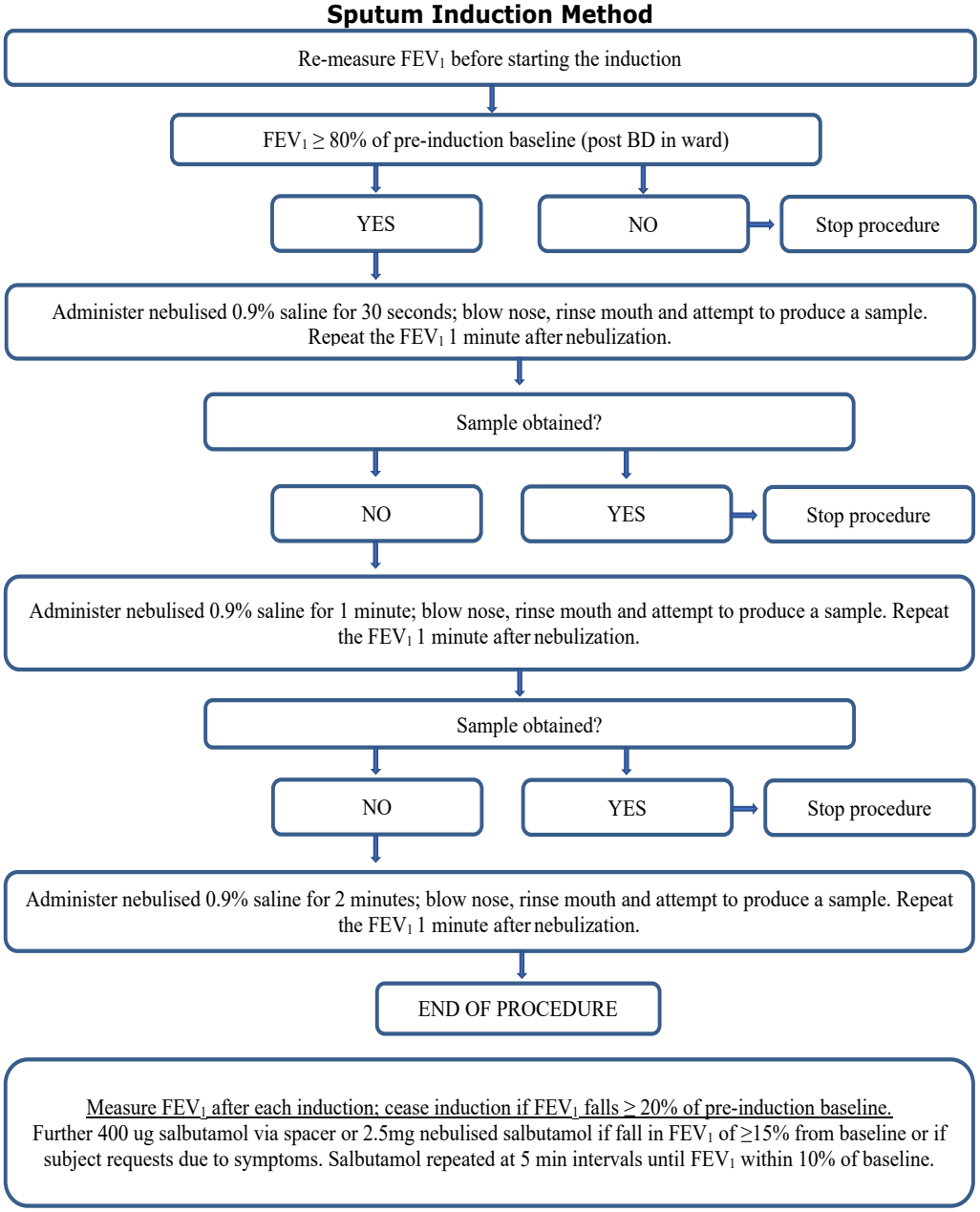
- Any features of life-threatening attack on **admission**/**currently** as per BTS guideline: (PEF <33% best or predicted, SpO2 <92%, PaO2 <8 kPa, 'normal' PaCO2 (4.6–6.0 kPa), Altered conscious level, Exhaustion, Arrhythmia, Hypotension, Cyanosis, Silent chest, Poor respiratory effort)
- Requiring supplemental O2 to maintain SpO2 ≥94%
- Patient, clinical team or investigator opinion that patient unable to tolerate procedure

*If patient had salbutamol within 2 hours of FIRST FEV_1, consider FIRST FEV_1 value as a post BD (BASELINE)

Nottingham Respiratory Research Unit
• Asthma • COPD • ILD • Lung Infection •

Nottingham University Hospitals NHS
NHS Trust

Sputum Induction Method



Measure FEV_1 after each induction; cease induction if FEV_1 falls $\geq 20\%$ of pre-induction baseline.
Further 400 ug salbutamol via spacer or 2.5mg nebulised salbutamol if fall in FEV_1 of $\geq 15\%$ from baseline or if subject requests due to symptoms. Salbutamol repeated at 5 min intervals until FEV_1 within 10% of baseline.

Sputum Induction Worksheet

Study Name: APEX3 Study ID: _____ Visit Number: _____ Date/Time: _____/____

1. Spirometry- Full Reversibility Procedure	
FEV_1 Pre BD. Skip if BD given within 2 Hrs	**FEV_1 Post BD (Best result is the BASELINE)**
1	1
2	2
3	3
Recovery FEV_1 Best FEV_1 Pre BD x 0.90 Use post if BD given within 2 Hrs **90% of best FEV_1 pre/post BD: ……(L)**	**Best FEV_1 Post BD (baseline)= ……** **% predicted = ……..** Must be ≥ 1L or ≥ 40% of predicted to proceed with induction.

Time of last BD:……

2.5mg nebulised salbutamol time given……

Ensure Dr available at site Dr/Prof…….....

2. Safety Measures -Calculate FEV_1 Post BD Drop		
Safety criteria	**FEV_1 Result**	**Action for induction**
No Drop or <20% drop in baseline FEV_1	>	No Drop or Drop of **<20%** of FEV_1 **from BASELINE, continue the induction procedure.**
*15% Drop (BASELINE x 0.85) OR Participant request due to symptoms worsening	≤	Drop of **≥15%** of FEV_1 from **BASELINE, repeat 400 ug salbutamol via spacer or 2.5mg nebulised salbutamol at 5 min intervals until FEV_1 ≥ 90% of baseline:……**
20% Drop (BASELINE x 0.80)	≤	Drop of **>20%** of FEV_1 **from BASELINE, stop the induction procedure and repeat the salbutamol.**

3. Sputum Induction: Pre induction (safety check) FEV_1 ≥ 80% of baseline?……			
Start Time:…….	**1st Induction**	**2nd Induction**	**3rd Induction**
Saline concentration	0.9%	0.9%	0.9%
Duration of induction	30 seconds	1 minute	2 minutes
Blow nose/Rinse mouth/Cough for sputum; repeat FEV_1			
Safety Blow 1	FEV_1:	FEV_1:	FEV_1:
Safety Blow 2 **Indicate best blow (1 or 2)**	FEV_1:	FEV_1:	FEV_1:
Sputum sample obtained If yes procedure completed	**Y/N** Time:	**Y/N** Time:	**Y/N** Time:
Further 400 ug salbutamol via spacer or 2.5mg nebulised salbutamol if fall in FEV_1 of ≥15%* from baseline or if subject requests due to symptoms.			
Recovery FEV_1 Must be ≥ 90% of **BEST PRE BD FEV_1 before leaving department**. If not, repeat Salbutamol at 5 min intervals until FEV1 ≥ 90% of **BEST PRE BD FEV_1. If ok procedure completed.** *Optional if not returned to 90% Pre BD value			
1. FEV_1: ……	**2. FEV_1: ……**	**3. FEV_1: ……**	
4. FEV_1: ……	**5. FEV_1: ……**	**6. FEV_1: ……**	

4. End of Procedure Safety
Document any adverse events. If the participant doesn't recover after 30 minutes, contact a doctor for advice. If the patient feels well the procedure is complete and patient can leave the department.

Induction completed by Name: Sign:
Date: Time:

Appendix E: APEX3- Cohort overview and clinical characteristics-summary of missing data.

Table 87. Demographic characteristics-missing data.

Demographics	N= 97
	N (%)
Age	0 (0%)
Gender	0 (0%)
Ethnicity	0 (0%)

Table 88. Medical history and comorbidities-missing data.

Medical history and comorbidities	N= 97
	N (%)
Previously diagnosed asthma	0 (0%)
Atopic comorbidities	0 (0%)
Significant comorbidities	0 (0%)
Airway diseases	0 (0%)
Asthma Mimics	0 (0%)
Smoking history	0 (0%)
Latex/alcohol allergy	0 (0%)
Medication allergies	0 (0%)

Table 89. Asthma specific history-missing data.

Previously diagnosed asthma	n= 87
Asthma-specific history	N (%)
Age onset	0 (0%)
Years with asthma	0 (0%)
Family history of asthma	0 (0%)
Perceived allergies	0 (0%)
Has asthma action plan	0 (0%)
Has peak flow meter	0 (0%)
Knows how to respond to a low PEFR	0 (0%)
Pet ownership	0 (0%)

Table 90. Asthma treatment-missing data.

Previously diagnosed asthma	n= 87
Asthma Treatment	N (%)
Not on asthma treatment	0 (0%)
SABA only	0 (0%)
On GINA treatment Step	0 (0%)
Biologics	0 (0%)
Low-dose maintenance OCS	0 (0%)
Biologics or Low-dose maintenance OCS	0 (0%)

Table 91. Asthma attack history-missing data.

Previously diagnosed asthma	n= 87
Asthma attack history	N (%)
Number of unscheduled visits (A&E, GP, etc) last 12 months	0 (0%)
>1 unscheduled visits (A&E, GP, etc) last 12 months	0 (0%)
Number of OCS courses last 12 months	0 (0%)
>1 OCS courses last 12 months	0 (0%)
Number of AAs last 12 months	0 (0%)
>1 AAs last 12 months	0 (0%)
Previous admissions for asthma (ever)	0 (0%)
• *Number of previous admissions (ever)*	0 (0%)
• *Number of NON-ITU admissions* last 12 months	0 (0%)
• *>1 NON-ITU admissions* last 12 months	0 (0%)
• *Previous ITU admission (ever)*	0 (0%)
• *Previously Intubated (ever)*	0 (0%)

Table 92. Characteristics of admissions identified-missing data.

Admission characteristics	N= 97
	N (%)
Time to recruitment in hours	0 (0%)
Length of stay in days	0 (0%)
Different primary discharge diagnosis	0 (0%)
Secondary diagnosis with potential relevance to presenting symptoms	0 (0%)
Pre-admission treatment with oral corticosteroids	0 (0%)
Pre-recruitment nebuliser	0 (0%)
On systemic anti-inflammatory at the time of study recruitment	0 (0%)

Table 93. Lung function measures-missing data.

Lung function measures	N= 97
	N (%)
Spirometry	12 (12.4%)
Forced oscillation	3 (3.1%)
Breath sounds	0 (0%)

Table 94. Inflammatory measures-missing data.

Inflammatory measures	N= 97
	N (%)
Blood eosinophil count	2 (2.1%)
FeNO	6 (6.2%)
Sputum inflammatory markers	45 (46.4%)

Table 95. Cytokines profiling-missing data.

Cytokine profiles	N= 97
T2-high cytokines	N (%)
• IL-5	60 (61.9)
• IL-13	32 (32.9)
• IL-4	20 (20.6)
Epithelial derived cytokines	
• IL-33	23 (23.7)
• TSLP	20 (20.6)
Th17 cytokines	
• IL-17	20 (20.6)
Pro-inflammatory cytokines	
• IL-8	32 (32.9)
• IL-1β	20 (20.6)
• IL-6	20 (20.6)
• TNF-α	20 (20.6)
Interferons	
• IFN-α	20 (20.6)
• IFN-γ	21 (21.6)
• IFN-β	52 (53.6)

Table 96. Evidence of infection-mission data.

Infection measures	N= 97
	N (%)
Sputum MC&S	41 (42.3%)
PCR throat swab	53 (54.6%)
CRP	1 (1.0%)
BNC	2 (2.1%)

Table 97. Symptoms scores and Extra-pulmonary traits-missing data.

Symptoms scores and Extra-pulmonary traits	N= 97
	N (%)
AAQLQ	2 (2.1%)
SNOT-22	35 (36.1%)
GERDQ	26 (26.8%)
PVCDQI	6 (6.2%)
The Nijmegen Questionnaire	3 (3.1%)
HADS: Anxiety	37 (38.1%)
HADS: Depression	41 (42.3%)
BMI	0 (0%)
Vitamin D	2 (2.1%)
MARS	13 (13.4%)
MPR	17 (17.5%)
Inhaler technique assessment	30 (30.9%)

Appendix F: APEX3- Comparison between Likely Acute Asthma-Related Admissions (LAARA, n= 64) and Likely Not Acute-Asthma Admissions (LINAA, n= 19)- summary of missing data.

Table 98. Demographic characteristics of patients with and without evidence of acute asthma-missing data.

Demographics	N= 83 (100%)	LAARA (n= 64, 77.1%)	LINAA (n= 19, 22.9%)
	N (%)		
Age	0 (0%)	0 (0%)	0 (0%)
Gender	0 (0%)	0 (0%)	0 (0%)
Ethnicity	0 (0%)	0 (0%)	0 (0%)

Table 99. Medical history and comorbidities in patients with and without evidence of acute asthma-missing data.

Medical history and comorbidities	N= 83 (100%)	LAARA (n= 64, 77.1%)	LINAA (n= 19, 22.9%)
	N (%)		
Previously diagnosed asthma	0 (0%)	0 (0%)	0 (0%)
Atopic comorbidities	0 (0%)	0 (0%)	0 (0%)
Significant comorbidities	0 (0%)	0 (0%)	0 (0%)
Airway diseases	0 (0%)	0 (0%)	0 (0%)
Asthma Mimics	0 (0%)	0 (0%)	0 (0%)
Smoking history	0 (0%)	0 (0%)	0 (0%)
Latex/alcohol allergy	0 (0%)	0 (0%)	0 (0%)
Medication allergies	0 (0%)	0 (0%)	0 (0%)

Table 100. Asthma-specific history in patients with and without evidence of acute asthma-missing data.

Previously diagnosed asthma	N= 74	LAARA (n= 55)	LINAA (n= 19)
Asthma-specific history	N (%)		
Age onset	0 (0%)	0 (0%)	0 (0%)
Years with asthma	0 (0%)	0 (0%)	0 (0%)
Family history of asthma	0 (0%)	0 (0%)	0 (0%)
Perceived allergies	0 (0%)	0 (0%)	0 (0%)
Has asthma action plan	0 (0%)	0 (0%)	0 (0%)
Has peak flow meter	0 (0%)	0 (0%)	0 (0%)
Knows how to respond to a low PEFR	0 (0%)	0 (0%)	0 (0%)
Pet ownership	0 (0%)	0 (0%)	0 (0%)

Table 101. Asthma treatment in patients with and without evidence of acute asthma- missing data.

Previously diagnosed asthma	N= 74	LAARA (n= 55)	LINAA (n= 19)
Asthma treatment	**N (%)**		
Not on asthma treatment	0 (0%)	0 (0%)	0 (0%)
SABA only	0 (0%)	0 (0%)	0 (0%)
On GINA treatment Step	0 (0%)	0 (0%)	0 (0%)
Biologics	0 (0%)	0 (0%)	0 (0%)
Low-dose maintenance OCS	0 (0%)	0 (0%)	0 (0%)
Biologics or Low-dose maintenance OCS	0 (0%)	0 (0%)	0 (0%)

Table 102. Asthma attack history in patients with and without evidence of acute asthma- missing data.

Previously diagnosed asthma	N= 74	LAARA (n= 55)	LINAA (n= 19)
Asthma-attack history	**N (%)**		
Number of unscheduled visits (A&E, GP, etc) last 12 months	0 (0%)	0 (0%)	0 (0%)
>1 unscheduled visits (A&E, GP, etc) last 12 months	0 (0%)	0 (0%)	0 (0%)
Number of OCS courses last 12 months	0 (0%)	0 (0%)	0 (0%)
>1 OCS courses last 12 months	0 (0%)	0 (0%)	0 (0%)
Number of AAs last 12 months	0 (0%)	0 (0%)	0 (0%)
>1 AAs last 12 months	0 (0%)	0 (0%)	0 (0%)
Previous admissions for asthma	0 (0%)	0 (0%)	0 (0%)
• *Number of previous admissions (ever)*	0 (0%)	0 (0%)	0 (0%)
• *Number of NON-ITU admissions* last 12 months	0 (0%)	0 (0%)	0 (0%)
• *>1 NON-ITU admissions* last 12 months	0 (0%)	0 (0%)	0 (0%)
• *Previous ITU admission (ever)*	0 (0%)	0 (0%)	0 (0%)
• *Previously Intubated (ever)*	0 (0%)	0 (0%)	0 (0%)

Table 103. Admission characteristics in patients with and without evidence of acute asthma-missing data.

Admission characteristics	N= 83 (100%)	LAARA (n= 64, 77.1%)	LINAA (n= 19, 22.9%)
	N (%)		
Time to recruitment (in hours)	0 (0%)	0 (0%)	0 (0%)
Length of stay in days	0 (0%)	0 (0%)	0 (0%)
Different Primary discharge diagnosis	0 (0%)	0 (0%)	0 (0%)
Secondary Diagnosis	0 (0%)	0 (0%)	0 (0%)
Pre-admission OCS	0 (0%)	0 (0%)	0 (0%)
Pre-recruitment nebuliser	0 (0%)	0 (0%)	0 (0%)
On systemic anti-inflammatory	0 (0%)	0 (0%)	0 (0%)

Table 104. Lung function measures in patients with and without evidence of acute asthma-missing data.

Lung function measures	N= 83 (100%)	LAARA (n= 64, 77.1%)	LINAA (n= 19, 22.9%)
	N (%)		
Spirometry	0 (0%)	0 (0%)	0 (0%)
Forced oscillation	0 (0%)	0 (0%)	0 (0%)
Breath sounds	0 (0%)	0 (0%)	0 (0%)

Table 105. Inflammatory measures in patients with and without evidence of acute asthma-missing data.

Inflammatory measures	N= 83 (100%)	LAARA (n= 64, 77.1%)	LINAA (n= 19, 22.9%)
	N (%)		
Blood eosinophil count	2 (2.4%)	1 (1.6%)	1 (5.3%)
FeNO	3 (3.6%)	3 (4.7%)	0 (0%)
Sputum inflammatory markers	38 (45.8%)	29 (45.3%)	9 (47.4%)

Table 106. Cytokines profiling in patients with and without evidence of acute asthma-missing data.

Cytokine profiles	N= 83 (100%)	LAARA (n= 64, 77.1%)	LINAA (n= 19, 22.9%)
T2-high cytokines	N (%)		
• IL-5	51 (61.5%)	40 (62.5%)	11 (57.9%)
• IL-13	28 (34.9%)	25 (39.1%)	3 (15.8%)
• IL-4	16 (19.3%)	15 (23.4%)	1 (5.3%)
Epithelial derived cytokines			
• IL-33	19 (22.9%)	18 (28.1%)	1 (5.3%)
• TSLP	16 (19.3%)	15 (23.4%)	1 (5.3%)
Th17 cytokines			
• IL-17	16 (19.3%)	15 (23.4%)	1 (5.3%)
Pro-inflammatory cytokines			
• IL-8	27 (32.5%)	22 (34.4%)	5 (26.3%)
• IL-1β	16 (19.3%)	15 (23.4%)	1 (5.3%)
• IL-6	16 (19.3%)	15 (23.4%)	1 (5.3%)
• TNF-α	16 (19.3%)	15 (23.4%)	1 (5.3%)
Interferons			
• IFN-α	16 (19.3%)	15 (23.4%)	1 (5.3%)
• IFN-γ	17 (20.5%)	16 (25.0%)	1 (5.3%)
• IFN-β	44 (53.0%)	33 (51.6%)	11 (57.9%)

Table 107. Evidence of infection in patients with and without evidence of acute asthma -mission data.

Infection status	N= 83 (100%)	LAARA (n= 64, 77.1%)	LINAA (n= 19, 22.9%)
	N (%)		
Sputum MC&S	35 (42.2)	30 (46.9)	5 (26.3)
PCR throat swab	46 (55.4)	34 (53.1)	12 (63.2)
CRP	1 (1.2)	0 (0)	1 (5.3)
BNC	2 (2.4)	1 (1.6)	1 (5.3)

Table 108. Symptoms scores and Extra-pulmonary traits in patients with and without evidence of acute asthma -missing data.

Symptoms scores and Extra-pulmonary traits	N= 83 (100%)	LAARA (n= 64, 77.1%)	LINAA (n= 19, 22.9%)
	N (%)		
AAQLQ	1 (1.2)	0 (0)	1 (5.3)
SNOT-22	31 (37.3)	22 (34.4)	9 (47.4)
GERDQ	23 (27.7)	16 (25.0)	7 (36.8)
PVCDQI	5 (6.0)	4 (6.3)	1 (5.3)
The Nijmegen Questionnaire	2 (2.4)	2 (3.1)	0 (0)
HADS: Anxiety	33 (39.8)	24 (37.5)	9 (47.4)
HADS: Depression	38 (5.8)	28 (43.8)	10 (52.6)
BMI	0 (0)	0 (0)	0 (0)
Vitamin D	2 (2.4)	2 (3.1)	0 (0)
MARS	11 (13.3)	11 (17.2)	0 (0)
MPR	15 (18.1)	13 (20.3)	2 (10.5)
Inhaler technique assessment	26 (31.3)	22 (34.3)	4 (21.1)

Appendix G: APEX3- Comparison between Likely Acute Asthma-Related Admissions (LAARA, n= 64) and Non-Asthma-Related Admissions (NARA, n= 9)

Summary of findings

Demographics and medical history

The NARA group were likely to be younger patients (Table 109) with slightly increased comorbidities and asthma mimics (Table 110); concurrent diagnosed airway diseases were similar between the groups. The proportion of patients with previous asthma diagnosis was not different between the groups although all those in the NARA had previously diagnosed asthma (Table 110). All those from the NARA were on asthma medications compared to 80% in the LAARA group; in patients on asthma treatment, a significantly increased number of those in the NARA were on step 5 GINA treatment step (Table 112). The median number of unscheduled visits (A&E, GP, etc), OCS courses, and AAs in the 12 months preceding study recruitment were significantly higher in the NARA compared to the LAARA (Table 113). The proportion of patients with >1 unscheduled visit, >1 OCS course, and >1 AA in the 12 months prior to study recruitment tended to be higher in NARA compared to LAARA. The median number of non-ITU admissions for asthma in the year prior to study recruitment was significantly higher in the NARA (Table 113).

Admission characteristics (visit 1)

Admission characteristics, including time from hospital admission to study recruitment, length of hospital stay, different primary diagnoses, and secondary diagnoses relevant to presenting symptoms were not different between the groups (Table 114).

Lung function measures and breath sounds

As expected, as sub-groups were classified based on the presence of evidence of airflow obstruction, mean FEV_1%, FEV_1/FVC were significantly increased (Table 115), and mean R5 was significantly decreased (Table 116), in the NARA compared to the LAARA. Similarly, the proportion of those with a clear breath sound was significantly increased in the NARA compared to the LAARA (Table 118).

Inflammatory profile

Again, as expected, as inflammatory measures were used to classify subgroups, median BEC and FeNO values were higher in the LAARA compared to the NARA (Table 119). The proportion of those with raised $T2^{APEX3}$ markers was higher in the LAARA compared to the NARA. Cytokine levels were similar between the groups (Table 120).

Evidence of infections

The proportion of those with infection (Positive sputum MC&S, PCR throat swab, or increased CRP or BNC) was slightly increased in the LAARA group, although this was not significantly different (Table 121).

Symptoms score

The overall mean AAQLQ score was not different between the groups. AAQLQ symptoms and emotional scores were also similar between the groups (Table 122).

Extra-pulmonary traits

Extrapulmonary comorbidity questionnaire scores were similar between the groups (Table 123). The median BMI and the proportion of those with BMI ≥25 (overweight) tended to be slightly increased in the NARA (Table 124). Medication possession ratio tended to higher in the NARA compared to the LAARA (Table 125).

Table 109. Demographic characteristics of patients with and without evidence of asthma pathology.

Demographics	N= 73 (100%)	LAARA (n= 64, 87.7%)	NARA (n= 9, 12.3%)	P value
	N (%) unless otherwise stated			
Age (Mean ± SD) (years)	42.8 ± 16.1	43.8 ± 16.7	35.7 ± 8.3	**0.03**
Gender				
• *Male*	22 (30.1)	19 (29.7)	3 (33.3)	1
• *Female*	51 (69.9)	45 (70.3)	6 (66.7)	
Ethnicity				
• *White/Caucasian*	52 (71.2)	44 (68.8)	8 (88.9)	0.89
• *Black/Black British*	7 (9.6)	7 (10.9)	0 (0)	
• *Asian/Asian British*	11 (15.1)	10 (15.6)	1 (11.1)	
• *Other*	3 (4.1)	3 (4.7)	0 (0)	

Table 110. Medical history and comorbidities in patients with and without evidence of asthma pathology.

Medical history and comorbidities	N= 73 (100%)	LAARA (n= 64, 87.7%)	NARA (n= 9, 12.3%)	P value
	N (%) unless otherwise stated			
Previously diagnosed asthma	64 (87.7)	55 (85.9)	9 (100)	0.59
Atopic comorbidities	28 (38.4)	26 (40.6)	2 (22.2)	0.47
• *Eczema*	13 (17.8)	12 (18.8)	1 (11.1)	1
• *Rhinitis*	21 (28.8)	19 (29.7)	2 (22.2)	1
Significant comorbidities	56 (76.7)	47 (73.4)	9 (100)	0.10
• *Depression*	34 (46.6)	28 (43.8)	6 (66.7)	0.28
• *Anxiety*	29 (39.7)	24 (37.5)	5 (55.6)	0.47
• *Nasal polyps*	3 (4.1)	2 (3.1)	1 (11.1)	0.33
• *GERD*	20 (27.4)	15 (23.4)	5 (55.6)	0.10
• *Hypertension*	12 (16.4)	11 (17.2)	1 (11.1)	1
• *Obesity (BMI ≥30)*	33 (45.2)	28 (43.8)	5 (55.6)	0.51
• BMI (Mean ± SD)	30.6 ± 8.6	30.3 ± 8.9	33.1 ± 5.8	0.23
Airway diseases	6 (8.2)	5 (7.8)	1 (11.1)	0.56
• *COPD*	1 (1.4)	1 (1.6)	0 (0)	1
• *Sleep apnoea*	4 (5.5)	3 (4.7)	1 (11.1)	0.42
• *EDAC*	1 (1.4)	1 (1.6)	0 (0)	1
Asthma Mimics	5 (6.9)	3 (4.7)	2 (22.2)	0.11
• *ILO*	3 (4.1)	2 (3.1)	1 (11.1)	0.33
• *Dysfunctional breathing*	1 (1.4)	0 (0)	1 (11.1)	0.12
• *EDAC*	1 (1.4)	1 (1.6)	0 (0)	1
Smoking history				
• *Never*	41 (56.2)	36 (56.3)	5 (55.6)	0.71
• *Current*	15 (20.6)	14 (21.9)	1 (11.1)	
• *Ex smoker*	17 (23.3)	14 (21.9)	3 (33.3)	
Latex/alcohol allergy	5 (6.9)	4 (6.3)	1 (11.1)	0.49
Medication allergies	26 (35.6)	24 (37.5)	2 (22.2)	0.48

GERD: Gastroesophageal reflux disease. BMI: Body Mass Index. COPD: Chronic obstructive airway disease. EDAC: Excessive dynamic airway collapse. ILO: Inducible laryngeal obstruction.

Table 111. Asthma-specific history in patients with and without evidence of asthma pathology.

Previously diagnosed asthma	N= 64	LAARA (n= 55)	NARA (n= 9)	P value
Asthma-specific history	**N (%) unless stated otherwise**			
Age onset (in years) Median (IQR)	10 (4 – 25.5)	10 (4 – 25)	6 (5 – 25)	0.65
Years with asthma (Mean ± SD)	24.2 ± 13.9	24.8 ± 14.2	20.8 ± 12.9	0.38
Family history of asthma	32 (50.0)	29 (52.7)	3 (33.3)	0.47
Perceived allergies	51 (79.7)	45 (81.8)	6 (66.7)	0.37
• Animals (Cats, Dogs)	24 (37.5)	22 (40.0)	2 (22.2)	0.46
• Inhaled allergens (pollen, house dust mites)	49 (76.6)	43 (78.2)	6 (66.7)	0.43
• Grass, Trees	27 (42.2)	22 (40.0)	5 (55.6)	0.47
• Other: Mold, Seafood, Nuts	3 (4.6)	2 (3.6)	1 (11.1)	0.37
• Aspirin allergy	11 (17.2)	10 (18.2)	1 (11.1)	1
Has asthma action plan	20 (31.3)	16 (29.1)	4 (44.4)	0.44
Has peak flow meter	48 (75.0)	40 (72.7)	8 (88.9)	0.43
Knows how to respond to a low PEFR	34 (53.1)	27 (49.1)	7 (77.8)	0.16
Pet ownership	28 (43.8)	22 (40.0)	6 (66.7)	0.16

PEFR: peak expiratory flow rate.

Table 112. Asthma treatment in patients with and without evidence of asthma pathology.

Previously diagnosed asthma	N= 64	LAARA (n= 55)	NARA (n= 9)	P value
Asthma treatment	**N (%)**			
Not on asthma treatment	8 (12.5)	8 (14.6)	0 (0)	0.59
SABA only	3 (4.7)	3 (5.5)	0 (0)	1
On GINA treatment Step	53 (82.8)	44 (80.0)	9 (100)	0.34
• *Step 1*	1 (1.9)	0 (0)	1 (11.1)	**0.04**
• *Step 2*	3 (5.7)	3 (6.8)	0 (0)	
• *Step 3*	8 (15.1)	6 (13.6)	2 (22.2)	
• *Step 4*	23 (43.4)	22 (50.0)	1 (11.1)	
• *Step 5*	18 (33.9)	13 (29.6)	5 (55.6)	
Biologics	2 (3.8)	2 (4.6)	0 (0)	1
Low-dose maintenance OCS	2 (3.8)	1 (2.3)	1 (11.1)	0.31
Biologics or Low-dose maintenance OCS	4 (7.6)	3 (6.8)	1 (11.1)	0.54

SABA: Short-Acting Beta-Agonists. GINA: Global initiative for asthma. OCS: Oral corticosteroids.

Table 113. Asthma attack history in patients with and without evidence of asthma pathology.

Previously diagnosed asthma	N= 64	LAARA (n= 55)	NARA (n= 9)	P value
Asthma-attack history	N (%) unless stated otherwise			
Number of unscheduled visits (A&E, GP, etc) last 12 months Median (IQR)	2.5 (0 – 5)	2 (0 – 4)	6 (2 – 10)	**0.02**
>1 unscheduled visits (A&E, GP, etc) last 12 months	39 (60.9)	31 (56.4)	8 (88.9)	0.07
Number of OCS courses last 12 months Median (IQR)	2 (0 – 4)	1 (0 – 4)	5 (2 – 6)	**0.03**
>1 OCS courses last 12 months	33 (51.6)	26 (47.3)	7 (77.8)	0.14
Number of AAs last 12 months Median (IQR)	2 (0 – 4.3)	2 (0 – 4)	5 (2 – 6)	**0.04**
>1 AAs last 12 months	39 (60.9)	31 (56.4)	8 (88.9)	0.07
Previous admissions for asthma	47 (73.4)	41 (74.6)	6 (66.7)	0.69
• *Number of previous admissions (ever)* Median (IQR)	3 (1 – 7)	3 (1 – 7)	4 (2.3 – 31.3)	0.42
• *Number of NON-ITU admissions* last 12 months Median (IQR)	1 (0 – 1.5)	0 (0 – 1)	1.5 (1 – 2.8)	**0.02**
• *>1 NON-ITU admissions* last 12 months	12 (25.5)	9 (21.9)	3 (50.0)	0.16
• *Previous ITU admission (ever)*	12 (25.5)	10 (24.4)	2 (33.3)	0.64
• *Previously Intubated (ever)*	4 (8.5)	3 (7.3)	1 (16.7)	0.43

OCS: Oral corticosteroids. ITU: Intensive Treatment Unit.

Table 114. Admission characteristics in patients with and without evidence of asthma pathology.

Admission characteristics	N= 73 (100%)	LAARA (n= 64, 87.7%)	NARA (n= 9, 12.3%)	P value
	N (%) unless stated otherwise			
Time to recruitment (in hours) Median (IQR)	9.3 (2.7 – 15.0)	7.9 (2.6 – 15.0)	13.0 (7.0 – 14.7)	0.40
Length of stay in days Median (IQR)	3 (1 – 4)	2 (1 – 4)	4 (3 – 6)	0.17
Different Primary discharge diagnosis*	7 (9.6)	7 (10.9)	0 (0)	0.59
Secondary Diagnosis**	8 (10.9)	6 (9.4)	2 (22.2)	0.25
Pre-admission OCS	16 (21.9)	13 (20.3)	3 (33.3)	0.40
Pre-recruitment nebuliser	66 (90.4)	58 (90.6)	8 (88.9)	0.79
On systemic anti-inflammatory†	19 (26.0)	15 (23.4)	4 (44.4)	0.23

**Lower respiratory tract infections, inducible laryngeal obstruction, community-acquired pneumonia, influenza A infection, viral bronchitis, eosinophilic pneumonia. **Potentially relevant to presenting symptoms: Long covid, viral induced wheeze, type 2 respiratory failure, infective exacerbation of asthma, undiagnosed COPD, an element of upper airway wheeze and anxiety, congestive cardiac failure/obesity hyperventilation syndrome, anxiety/hyperventilation, upper airway noise, possible dysfunctional breathing, multifactorial shortness of breath. †At the time of study recruitment: Low-dose OCS, biologics, or pre-admission treatment with OCS.*

Table 115. Spirometry parameters in patients with and without evidence of asthma pathology.

Spirometry parameters	N= 73 (100%)	LAARA (n= 64, 87.7%)	NARA (n= 9, 12.3%)	P value
	N (%) unless stated otherwise			
On admission FEV_1% (Mean ± SD)	63.0 ± 22.0	58.9 ± 19.4	92.6 ± 16.6	**<0.001**
On admission FVC% (Mean ± SD)	77.5 ± 19.5	75.0 ± 18.5	95.4 ± 18.5	**0.01**
On admission FEV_1/FVC (Mean ± SD)	66.1 ± 14.1	64.0 ± 13.7	80.9 ± 3.1	**<0.001**
On admission PEFR% Median (IQR)	79.0 (61.7 – 93.8)	74.8 (59.0 – 92.9)	90.6 (84.6 – 100.8)	**0.02**
FEV_1 <80%	58 (79.5)	56 (87.5)	2 (22.2)	**<0.001**
FVC <80%	42 (57.5)	40 (62.5)	2 (22.2)	**0.02**
FEV_1 >80% and FVC <80%	1 (1.4)	0 (0)	1 (11.1)	0.12
FEV_1/FVC >70%	25 (34.3)	16 (25.0)	9 (100)	
PEFR <75%	33 (45.2)	32 (50.0)	1 (11.1)	**0.04**

Table 116. Forced oscillation technique parameters in patients with and without evidence of asthma pathology.

FOT parameters	N= 73 (100%)	LAARA (n= 64, 87.7%)	NARA (n= 9, 12.3%)	P value
	N (%) unless stated otherwise			
On admission R5 (Mean ± SD)	5.9 ± 2.4	6.2 ± 2.3	3.6 ± 0.9	<0.001
R5 <5	30 (41.1)	21 (32.8)	9 (100)	

Table 117. Small airway involvement in patients with and without evidence of asthma pathology.

Small airway disease	N= 73 (100%)	LAARA (n= 64, 87.7%)	NARA (n= 9, 12.3%)	P value
	N (%) unless stated otherwise			
On admission $FEF_{25-75\%}$ (Mean ± SD)	43.8 ± 28.2	37.7 ± 23.9	87.5 ± 13.8	<0.001
On admission R5-R20 Median (IQR)	1.6 (0.53 – 2.66)	2.0 (0.82 – 3.0)	0.3 (0.02 – 0.54)	<0.001
$FEF_{25-75\%}$ <65%	53 (72.6)	53 (82.8)	0 (0)	<0.001
R5-R20 >1	46 (63.0)	45 (70.3)	1 (11.1)	<0.001
Small airway disease*	6 (8.2)	6 (9.4)	0 (0)	1

**Defined as per Lipworth's criteria: Preserved FEV_1 (>80%) and (FEF_{25-75} <60% OR R5-20 >1 $cmH_2O/L/s$).*

Table 118. Breath sounds in patients with and without evidence of asthma pathology.

Breath sounds	N= 73 (100%)	LAARA (n= 64, 87.7%)	NARA (n= 9, 12.3%)	P value
	N (%)			
Breath sounds: Clear	31 (42.5)	24 (37.5)	7 (77.8)	0.03
Breath sounds: Wheezing	40 (54.8)	38 (59.4)	2 (22.2)	0.07
Breath sounds: Other	2 (2.7)	2 (3.1)	0 (0)	1

Table 119. Inflammatory profiles in patients with and without evidence of asthma pathology.

Inflammatory profile	N= 73 (100%)	LAARA (n= 64, 87.7%)	NARA (n= 9, 12.3%)	P value
BEC Median (IQR) (n=72)	0.13 (0.04 – 0.38)	0.16 (0.05 – 0.43)	0.03 (0.1 – 0.12)	0.05
FeNO Median (IQR) (n=70)	20 (10.3 – 55.8)	26 (12 – 60)	10.0 (7.0 – 19.0)	**0.01**
SEC% Median (IQR) (n=38)	0.25 (0 – 3.9)	0.25 (0 – 4.4)	0.25 (0.13 – 0.25)	0.37
SNC% Median (IQR) (n=38)	87.4 (53.8 – 97.1)	88.5 (60.9 – 97.3)	39.0 (30.0 – 45.4)	**0.02**
SNC ≥65%	25 of 38 (65.8)	25 of 35 (71.4)	0 of 3 (0)	**0.03**
Raised $T2^{APEX3}$	38 (52.1)	38 (59.4)	0 (0)	**<0.001**
• BEC ≥0.4 $x10^9$/L	17 of 72 (23.6)	17 of 63 (26.9)	0 of 9 (0)	0.10
• FeNO ≥25 ppb	31 of 70 (44.3)	31 of 61 (50.8)	0 of 9 (0)	**0.003**
• SEC ≥3%	12 of 38 (31.6)	12 of 35 (34.3)	0 of 3 (0)	0.53
Raised $T2^{APEX3}$ /Neutrophils	50 (68.5)	50 (78.1)	0 (0)	**<0.001**
Normal $T2^{APEX3}$ /Neutrophils	23 (31.5)	14 (21.9)	9 (100.0)	
Raised $T2^{GINA}$	51(69.9)	48 (75.0)	3 (33.3)	**0.02**
• BEC ≥0.15 $x10^9$/L	34 of 72 (47.2)	32 of 63 (50.8)	2 of 9 (22.2)	0.16
• FeNO ≥20 ppb	38 of 70 (54.3)	36 of 61 (59.0)	2 of 9 (22.2)	0.07
• SEC ≥2%	14 of 38 (36.8)	14 of 35 (40.0)	0 of 3 (0)	0.28
Raised $T2^{GINA}$/Neutrophils	60 (82.2)	57 (89.1)	3 (33.3)	**<0.001**
Normal $T2^{GINA}$/Neutrophils	13 (17.8)	7 (10.9)	6 (66.7)	

BEC: Blood eosinophil count. FeNO: fractional exhaled nitric oxide. SEC: sputum eosinophils count. SNC: sputum neutrophils count.

Table 120. Cytokines profiling in patients with and without evidence of asthma pathology.

Cytokine profiles	N= 73 (100%)	LAARA (n= 64, 87.7%)	NARA (n= 9, 12.3%)	P value
T2-high cytokines	Median (IQR)			
• IL-5 Median (IQR) (n= 29)	2.8 (1.7 – 5.9)	3.1 (1.7 – 5.7)	2.8 (1.9 – 6.3)	0.93
• IL-13 Median (IQR) (n= 47)	244.9 (128.6 – 378.6)	244.9 (149.3 – 367.6)	112.2 (41.8 – 787.7)	0.31
• IL-4 Median (IQR) (n= 57)	43.8 (33.5 – 61.2)	44.4 (34.1 – 57.5)	36.0 (29.1 – 70.9)	0.69
Epithelial derived cytokines				
• IL-33 Median (IQR) (n= 54)	74.1 (49.2 – 105.4)	75.7 (49.2 – 122.5)	59.9 (53.6 – 85.8)	0.44
• TSLP Median (IQR) (n= 57)	1.5 (1.0 – 2.3)	1.7 (1.1 – 2.2)	1.1 (0.9 – 2.4)	0.69
Th17 cytokines				
• IL-17 Median (IQR) (n= 57)	381.4 (248.8 – 549.2)	381.3 (248.8 – 521.8)	377.6 (252.0 – 600.9)	0.96
Pro-inflammatory cytokines				
• IL-8 Median (IQR) (n= 48)	1459.4 (622.9 – 2602.2)	1459.4 (747.7 –2628.3)	1083.4 (548.4 – 1627.7)	0.26
• IL-1β Median (IQR) (n= 57)	47.6 (23.2 – 105.5)	46.6 (20.8 – 115.9)	60.9 (27.7 – 84.2)	0.77
• IL-6 Median (IQR) (n= 57)	20.7 (8.5 – 40.1)	21.7 (8.5 – 46.2)	22.4 (14.9 – 33.5)	0.9
• TNF-α Median (IQR) (n= 57)	13.6 (7.5 – 28.1)	13.6 (7.5 – 31.6)	12.7 (8.5 – 21.5)	0.79
Interferons				
• IFN-α Median (IQR) (n= 57)	10.4 (6.7 – 21.2)	10.4 (6.7 – 18.2)	15.8 (6.3 – 28.3)	0.61
• IFN-γ Median (IQR) (n= 56)	4.9 (2.9 – 7.6)	4.8 (3.2 – 7.4)	5.5 (2.6 – 10.9)	0.96
• IFN-β Median (IQR) (n= 35)	15.4 (4.0 – 20.3)	12.9 (3.9 – 19.0)	19.4 (13.7 – 26.8)	0.24

Table 121. Evidence of infection assessed on admission in patients with and without evidence of asthma pathology.

Infection status	N= 73 (100%)	LAARA (n= 64, 87.7%)	NARA (n= 9, 12.3%)	P value
	N (%)			
Positive sputum MC&S	4 of 41 (9.8)	4 of 34 (11.8)	0 of 7 (0)	1
Positive PCR throat swab	9 of 33 (27.3)	9 of 30 (30.0)	0 of 3 (0)	0.54
CRP (>10 mg/L)	27 of 73 (36.9)	25 of 64 (39.1)	2 of 9 (22.2)	0.49
BNC (>8 $x10^9$/L)	22 of 72 (30.1)	21 of 63 (33.3)	1 of 9 (11.1)	0.26
Overall positive infection status*	40 (54.8)	37 (57.8)	3 (33.3)	0.28

*MC&S: Sputum microscopy culture sensitivities. CRP: C-reactive protein. BNC: Blood neutrophil count. *Positive sputum MC&S, PCR throat swab, or increased CRP or BNC.*

Table 122. Acute asthma quality of life questionnaire assessed on admission in patients with and without evidence of asthma pathology.

AAQLQ score	N= 73 (100%)	LAARA (n= 64, 87.7%)	NARA (n= 9, 12.3%)	P value
	Mean ± SD			
AAQLQ overall score (n=72)	3.9 ± 1.5	3.9 ± 1.6	3.8 ± 0.5	0.82
• *Symptoms score*	3.9 ± 1.5	3.9 ± 1.6	4.3 ± 0.7	0.29
• *Emotional score*	3.8 ± 1.7	3.9 ± 1.7	3.4 ± 0.9	0.19

Table 123. Extrapulmonary comorbidity questionnaires assessed on admissions in patients with and without evidence of asthma pathology.

Extra-pulmonary comorbidity questionnaires	N= 73 (100%)	LAARA (n= 64, 87.7%)	NARA (n= 9, 12.3%)	P value
	N (%) unless stated otherwise			
SNOT-22 score (Mean ± SD) (n= 51)	44.9 ± 24.7	44.4 ± 25.0	48.9 ± 24.3	0.66
• Mild (8-20)	9 of 49 (18.4)	8 of 42 (19.1)	1 of 7 (14.3)	0.86
• Moderate (>20-50)	19 of 49 (38.8)	17 of 42 (40.5)	2 of 7 (28.6)	
• Severe (>50)	21 of 49 (42.9)	17 of 42 (40.5)	4 of 7 (57.1)	
• SNOT-22 >8 or Rhinitis diagnosis	70 (95.9)	61 (95.3)	9 (100)	1
GERDQ score Median (IQR) (n=52)	6 (6 – 7)	6 (6 – 7)	6.5 (6 – 8.5)	0.30
• GERDQ >8	6 of 52 (11.5)	5 of 48 (10.4)	1 of 4 (25.0)	0.39
• GERDQ >8 or GERD diagnosis	26 (35.6)	20 (31.3)	6 (66.7)	0.06
PVCDI score Median (IQR) (n=68)	3 (2 – 6)	3 (2 – 6)	4 (2 – 6)	0.62
• PVCDI score >3	33 of 68 (48.5)	29 of 60 (48.3)	5 of 8 (50.0)	1
• PVCDI score >3 or VCD diagnosis	36 (49.3)	31 (48.4)	5 (55.6)	0.74
Nijmegen score Median (IQR) (n=71)	20.0 (11.5 – 31.0)	19.5 (12.0 – 31.0)	21.0 (9.0 – 25.0)	0.48
• Nijmegen Index >23	29 of 71 (40.9)	26 of 62 (41.9)	3 of 9 (33.3)	0.72
• Nijmegen Index >23 or Dysfunctional breathing diagnosis	30 (41.1)	26 (40.6)	4 (44.4)	1
HADS anxiety score Median (IQR) (n=44)	4.0 (2.0 – 7.3)	4.0 (2.0 – 7.3)	3 (1.5 – 6.3)	0.59
• HADS anxiety score >11	7 of 44 (15.9)	6 of 40 (15.0)	1 of 4 (25.0)	0.51
• HADS anxiety score >11 or anxiety diagnosis	36 (49.3)	30 (46.9)	6 (66.7)	0.30
HADS depression score Median (IQR) (n=39)	3.0 (0.5 – 5.5)	3.0 (0 – 5.3)	5.0 (4.5 – 6.5)	0.18
• HADS depression score >11	0 of 39 (0)	0 of 36 (0)	0 of 3 (0)	
• HADS depression score >11 or depression diagnosis	34 (46.6)	28 (43.8)	6 (66.7)	0.29

SNOT-22: Sino-nasal outcome test-22 questionnaire. GERDQ: Gastroesophageal reflux disease questionnaire. PVCDI: Pittsburgh Vocal Cord Dysfunction Index. HADS: Hospital Anxiety and Depression Scale.

Table 124. Body mass index and vitamin D in patients with and without evidence of asthma pathology.

Other measures	N= 73 (100%)	LAARA (n= 64, 87.7%)	NARA (n= 9, 12.3%)	P value
	N (%) unless stated otherwise			
BMI Median (IQR)	29.4 (23.4 – 34.4)	28.1 (22.8 – 34.3)	31.6 (29.4 – 30.0)	0.16
BMI ranges				
• *Underweight (<18.5)*	1 (1.4)	1 (1.6)	0 (0)	0.26
• *Healthy range (18.5 – 24.9)*	19 (26.0)	19 (29.7)	0 (0)	
• *Overweight (25 – 29.9)*	20 (27.4)	16 (25.0)	4 (44.4)	
• *Obesity (30 – 39.9)*	22 (30.1)	19 (29.7)	3 (33.3)	
• *Severe obesity (≥40)*	11 (15.1)	9 (14.1)	2 (22.2)	
BMI ≥25 (overweight)	53 (72.6)	44 (68.8)	9 (100)	0.06
BMI ≥30 (obesity)	33 (45.2)	28 (43.8)	5 (55.6)	0.72
Vitamin D (Mean ± SD) (n=71)	49.9 ± 27.4	48.7 ± 28.1	58.7 ± 20.6	0.22
• *Vitamin D insufficiency (25-50 nmol/L)*	26 of 71 (36.6)	23 of 62 (37.1)	3 of 9 (33.3)	0.83
• *Vitamin D deficiency (<25 nmol/L)*	13 of 71 (18.3)	13 of 62 (20.9)	0 of 9 (0)	0.19

Table 125. Assessment of adherence in patients with and without evidence of asthma pathology.

Previously diagnosed asthma	N= 64	LAARA (n= 55)	NARA (n= 9)	P value
Adherence	N (%) unless stated otherwise			
MARS Median (IQR) (n= 62)	23.0 (20.3 – 25.0)	23.0 (20.0 – 25.0)	24.0 (23.0 – 24.0)	0.25
MPR Median (IQR) (n= 60)	58.0 (8.5 – 100.0)	50.0 (8.3 – 100.0)	100.0 (50.0 – 100.0)	0.05
MPR <75%	32 of 60 (53.3)	29 of 51 (56.9)	3 of 9 (33.3)	0.28
On GINA treatment Step	53 (82.8)	44 (80.0)	9 (100)	0.34
Inhaler technique (n= 50)				
• *Poor*	6 of 50 (12.0)	6 (14.3)	0 (0)	0.25
• *Good*	20 of 50 (40.0)	18 (42.9)	2 (25.0)	
• *Excellent*	24 of 50 (48.0)	18 (42.9)	6 (75.0)	
• *Total*	50	42	8	

MARS: Medication adherence report scale questionnaire. MPR: Medication Possession Ratio.

Appendix H: APEX3- Comparison between Likely Acute Asthma-Related Admissions (LAARA, n= 64) and Likely Out of Control Asthma (LOCA, n= 10)

Summary of findings

Demographics and medical history

Demographic characteristics were similar between the groups (Table 126). LOCA were likely to have atopic comorbidities, including eczema and rhinitis (Table 127). Significant comorbidities, airway diseases, and asthma mimics were all similar between the groups. Asthma-specific history (Table 128), asthma treatment (Table 129), and asthma attack history (Table 130) were also similar between the groups.

Admission characteristics (visit 1)

Time from hospital admission to study recruitment, length of hospital stay, different primary diagnoses, and secondary diagnoses relevant to presenting symptoms were all similar between the groups (Table 131).

Lung function measures and breath sounds

Again, as sub-groups were categorised based on airflow limitation, mean FEV_1%, FEV_1/FVC were significantly increased (Table 132), and mean R5 was significantly decreased (Table 133), in the LOCA compared to the LAARA. The proportion of those with a clear breath sound was significantly increased in the LOCA compared to the LAARA (Table 135).

Inflammatory profile

Again, as expected, as subgroups were categorised based on inflammatory profiles, median FeNO, and the proportion of those with increased markers of $T2^{APEX3}$ inflammation tended to be higher in the LOCA compared to the LAARA (Table 136). Median blood eosinophil count and the proportion of those with increased BEC levels (≥0.4 $x10^9$/L) were similar between the groups. Cytokine levels were largely similar between the groups apart from median IL-13 which was significantly increased in the LAARA compared to the LOCA (Table 137).

Evidence of infections

The proportion of those with infection (Positive sputum MC&S, PCR throat swab, or increased CRP or BNC) was similar between the groups (Table 138).

Symptoms score

There was no difference in AAQLQ scores between the groups (Table 139).

Extra-pulmonary traits

There was no difference in extrapulmonary comorbidity questionnaires between the groups (Table 140). The proportion of overweight and obese patients tended to be slightly higher in the LOCA group (Table 141).

Table 126. Demographic characteristics of patients with likely acute asthma compared to those with likely poorly controlled asthma.

Demographics	N= 74 (100%)	LAARA (n= 64, 86.5%)	LOCA (n= 10, 13.5%)	P value
	N (%) unless otherwise stated			
Age (Mean ± SD) (years)	44.1 ± 17.1	43.8 ± 16.7	46.0 ± 20.5	0.75
Gender				
• *Male*	23 (31.1)	19 (29.7)	4 (40)	0.49
• *Female*	51 (68.9)	45 (70.3)	6 (60.0)	
Ethnicity				
• *White/Caucasian*	51 (68.9)	44 (68.8)	7 (70.0)	1
• *Black/Black British*	8 (10.8)	7 (10.9)	1 (10.0)	
• *Asian/Asian British*	12 (16.2)	10 (15.6)	2 (20.0)	
• *Other*	3 (4.1)	3 (4.7)	0 (0)	

Table 127. Medical history and comorbidities in patients with likely acute asthma compared to those with likely poorly controlled asthma.

Medical history and comorbidities	N= 74 (100%)	LAARA (n= 64, 86.5%)	LOCA (n= 10, 13.5%)	P value
	N (%) unless otherwise stated			
Previously diagnosed asthma	65 (87.8)	55 (85.9)	10 (100)	0.35
Atopic comorbidities	34 (45.9)	26 (40.6)	8 (80.0)	**0.04**
• *Eczema*	17 (22.9)	12 (18.8)	5 (50.0)	**0.04**
• *Rhinitis*	26 (35.1)	19 (29.7)	7 (70.0)	**0.03**
Significant comorbidities	54 (72.9)	47 (73.4)	6 (60.0)	1
• *Depression*	32 (43.2)	28 (43.8)	4 (40.0)	1
• *Anxiety*	28 (37.8)	24 (37.5)	4 (40.0)	1
• *Nasal polyps*	3 (4.1)	2 (3.1)	1 (10.0)	0.36
• *GERD*	17 (22.9)	15 (23.4)	2 (20.0)	1
• *Hypertension*	12 (16.2)	11 (17.2)	1 (10.0)	1
• *Obesity (BMI ≥30)*	35 (47.3)	28 (43.8)	7 (70.0)	0.17
• BMI (Mean ± SD)	30.6 ± 8.6	30.3 ± 8.9	32.6 ± 6.1	0.31
Airway diseases	6 (8.1)	5 (7.8)	1 (10.0)	1
• *COPD*	1 (1.4)	1 (1.6)	0 (0)	1
• *Sleep apnoea*	4 (5.4)	3 (4.7)	1 (10.0)	0.45
• *EDAC*	1 (1.4)	1 (1.6)	0 (0)	1
Asthma Mimics	3 (4.1)	3 (4.7)	0 (0.0)	1
• *ILO*	2 (2.7)	2 (3.1)	0 (0.0)	1
• *Dysfunctional breathing*	0 (0)	0 (0)	0 (0)	
• *EDAC*	1 (1.4)	1 (1.6)	0 (0)	1
Smoking history				
• *Never*	41 (55.4)	36 (56.3)	5 (50.0)	0.42
• *Current*	15 (20.3)	14 (21.9)	1 (10.0)	
• *Ex smoker*	18 (24.3)	14 (21.9)	4 (40.0)	
• *Total*	74	64	10	
Latex/alcohol allergy	4 (5.4)	4 (6.3)	0 (0)	1
Medication allergies	28 (37.8)	24 (37.5)	4 (40.0)	1

Table 128. Asthma-specific history in patients with likely acute asthma compared to those with likely poorly controlled asthma.

Previously diagnosed asthma	N= 65	LAARA (n= 55)	LOCA (n= 10)	P value
Asthma-specific history	N (%) unless otherwise stated			
Age onset (in years) Median (IQR)	10.0 (0 – 27.0)	10 (4 – 25)	3.0 (0 – 28.3)	0.36
Years with asthma (Mean ± SD)	25.3 ± 14.1	24.5 ± 14.2	28.3 ± 14.1	0.41
Family history of asthma	32 (50.8)	29 (52.7)	4 (40.0)	0.40
Perceived allergies	55 (84.6)	45 (81.8)	10 (100)	0.33
• Animals (Cats, Dogs)	29 (44.6)	22 (40.0)	7 (70.0)	0.09
• Inhaled allergens (pollen, house dust mites)	53 (81.5)	43 (78.2)	10 (100)	0.19
• Grass, Trees	28 (43.1)	22 (40.0)	6 (60.0)	0.31
• Other: Mold, Seafood, Nuts	2 (3.1)	2 (3.6)	0 (0)	1
• Aspirin allergy	12 (18.5)	10 (18.2)	2 (20.0)	1
Has asthma action plan	19 (29.2)	16 (29.1)	3 (30.0)	1
Has peak flow meter	45 (69.2)	40 (72.7)	5 (50.0)	0.26
Knows how to respond to a low PEFR	32 (49.2)	27 (49.1)	5 (50.0)	1
Pet ownership	28 (43.1)	22 (40.0)	6 (60.0)	0.31

PEFR: peak expiratory flow rate.

Table 129. Asthma treatment in patients with likely acute asthma compared to those with likely poorly controlled asthma.

Previously diagnosed asthma	N= 65	LAARA (n= 55)	LOCA (n= 10)	P value
Asthma treatment	N (%)			
Not on asthma treatment	9 (13.9)	8 (14.6)	1 (10.0)	1
SABA only	4 (6.2)	3 (5.5)	1 (10.0)	0.49
On GINA treatment Step	52 (80.0)	44 (80.0)	8 (80.0)	1
• *Step 1*	0 (0)	0 (0)	0 (0)	0.49
• *Step 2*	4 (7.7)	3 (6.8)	1 (12.5)	
• *Step 3*	8 (15.4)	6 (13.6)	2 (25.0)	
• *Step 4*	26 (50.0)	22 (50.0)	4 (50.0)	
• *Step 5*	14 (26.9)	13 (29.6)	1 (12.5)	
Biologics	3 (5.8)	2 (4.6)	1 (12.5)	0.40
Low-dose maintenance OCS	1 (1.9)	1 (2.3)	0 (0)	1
Biologics or Low-dose maintenance OCS	4 (7.7)	3 (6.8)	1 (12.5)	1

SABA: Short-Acting Beta-Agonists. GINA: Global initiative for asthma. OCS: Oral corticosteroids.

Table 130. Asthma attack history in patients with likely acute asthma compared to those with likely poorly controlled asthma.

Previously diagnosed asthma	N= 65	LAARA (n= 55)	LOCA (n= 10)	P value
Asthma-attack history	N (%) unless stated otherwise			
Number of unscheduled visits (A&E, GP, etc) last 12 months Median (IQR)	2 (0 – 4)	2 (0 – 4)	1 (1 – 3)	0.81
>1 unscheduled visits (A&E, GP, etc) last 12 months	35(53.9)	31 (56.4)	4 (40.0)	0.49
Number of OCS courses last 12 months Median (IQR)	1 (0 – 4)	1 (0 – 4)	1 (0.3 – 2.8)	0.80
>1 OCS courses last 12 months	30 (46.2)	26 (47.3)	4 (40.0)	0.74
Number of AAs last 12 months Median (IQR)	2 (0 – 4)	2 (0 – 4)	1 (1 – 5)	0.97
>1 AAs last 12 months	35 (53.9)	31 (56.4)	4 (40.0)	0.49
Previous admissions for asthma	47 (72.3)	41 (74.6)	6 (60.0)	0.44
Number of previous admissions (ever) Median (IQR)	3 (1 – 7)	3 (1 – 7)	4.5 (1.8 – 8.8)	0.57
Number of NON-ITU admissions last 12 months Median (IQR)	0 (0 – 1)	0 (0 – 1)	0.5 (0 – 1)	0.69
>1 NON-ITU admissions last 12 months	9 (19.2)	9 (21.9)	0 (0)	0.58
Previous ITU admission (ever)	10 (21.3)	10 (24.4)	0 (0)	0.31
Previously Intubated (ever)	3 (6.4)	3 (7.3)	0 (0)	1

OCS: Oral corticosteroids. ITU: Intensive Treatment Unit.

Table 131. Admission characteristics in patients with likely acute asthma compared to those with likely poorly controlled asthma.

Admission characteristics	N= 74 (100%)	LAARA (n= 64, 86.5%)	LOCA (n= 10, 13.5%)	P value
	N (%) unless stated otherwise			
Time to recruitment (in hours) Median (IQR)	7.8 (2.8 – 14.0)	7.9 (2.6 – 15.0)	7.8 (6.8 – 8.5)	0.56
Length of stay in days Median (IQR)	2 (1 – 4)	2 (1 – 4)	1.5 (0 – 2)	0.13
Different Primary discharge diagnosis*	8 (10.8)	7 (10.9)	1 (10.0)	1
Secondary Diagnosis**	7 (9.5)	6 (9.4)	1 (10.0)	1
Pre-admission OCS	13 (17.6)	13 (20.3)	0 (0)	0.19
Pre-recruitment nebuliser	67 (90.5)	58 (90.6)	9 (90.0)	1
On systemic anti-inflammatory†	16 (21.6)	15 (23.4)	1 (10.0)	0.68

**Lower respiratory tract infections, inducible laryngeal obstruction, community-acquired pneumonia, influenza A infection, viral bronchitis, eosinophilic pneumonia. **Potentially relevant to presenting symptoms: Long covid, viral induced wheeze, type 2 respiratory failure, infective exacerbation of asthma, undiagnosed COPD, an element of upper airway wheeze and anxiety, congestive cardiac failure/obesity hyperventilation syndrome, anxiety/hyperventilation, upper airway noise, possible dysfunctional breathing, multifactorial shortness of breath. †At the time of study recruitment: Low-dose OCS, biologics, or pre-admission treatment with OCS.*

Table 132. Spirometry parameters in patients with likely acute asthma compared to those with likely poorly controlled asthma.

Spirometry parameters	N= 74 (100%)	LAARA (n= 64, 86.5%)	LOCA (n= 10, 13.5%)	P value
	N (%) unless stated otherwise			
On admission FEV_1% (Mean ± SD)	62.6 ± 20.7	58.9 ± 19.4	86.5 ± 10.1	**<0.001**
On admission FVC% (Mean ± SD)	77.0 ± 18.3	75.0 ± 18.5	89.9 ± 10.3	**0.001**
On admission FEV_1/FVC (Mean ± SD)	65.9 ± 13.8	64.0 ± 13.7	78.2 ± 5.3	**<0.001**
On admission PEFR% (Mean ± SD)	77.9 ± 24.3	74.9 ± 24.5	97.3 ± 9.8	**<0.001**
FEV_1 <80%	58 (78.4)	56 (87.5)	2 (20.0)	**<0.001**
FVC <80%	42 (56.7)	40 (62.5)	2 (20.0)	**0.01**
FEV_1 >80% and FVC <80%	0 (0)	0 (0)	0 (0)	[redacted]
FEV_1/FVC >70%	26 (35.1)	16 (25.0)	10 (100)	[redacted]
PEFR <75%	32 (43.2)	32 (50.0)	0 (0.0)	**0.03**

Table 133. Forced oscillation technique parameters in patients with likely acute asthma compared to those with likely poorly controlled asthma.

FOT parameters	N= 74 (100%)	LAARA (n= 64, 86.5%)	LOCA (n= 10, 13.5%)	P value
	N (%) unless stated otherwise			
On admission R5 (Mean ± SD)	5.8 ± 2.4	6.2 ± 2.3	3.5 ± 0.8	**<0.001**
R5 <5	31 (41.9)	21 (32.8)	10 (100)	

Table 134. Small airway involvement in patients with likely acute asthma compared to those with likely poorly controlled asthma.

Small airway disease	N= 74 (100%)	LAARA (n= 64, 86.5%)	LOCA (n= 10, 13.5%)	P value
	N (%) unless stated otherwise			
On admission $FEF_{25-75\%}$ (Mean ± SD)	43.3 ± 27.2	37.7 ± 23.9	79.0 ± 18.9	**<0.001**
On admission R5-R20 (Mean ± SD)	1.9 ± 1.6	2.1 ± 1.6	0.7 ± 0.7	**<0.001**
$FEF_{25-75\%}$ <65%	55 (74.3)	53 (82.8)	2 (20.0)	**<0.001**
R5-20 >1	48 (64.9)	45 (70.3)	3 (30.0)	**0.03**
Small airway disease*	8 (10.8)	6 (9.4)	2 (20.0)	0.29

**Defined as per Lipworth's criteria: Preserved FEV_1 (>80%) and (FEF_{25-75} <60% OR R5-20 >1 $cmH_2O/L/s$).*

Table 135. Breath sounds in patients with likely acute asthma compared to those with likely poorly controlled asthma.

Breath sounds	N= 74 (100%)	LAARA (n= 64, 86.5%)	LOCA (n= 10, 13.5%)	P value
	N (%)			
Breath sounds: Clear	32 (43.2)	24 (37.5)	8 (80.0)	**0.02**
Breath sounds: Wheezing	40 (54.8)	38 (59.4)	2 (20.0)	0.07
Breath sounds: Other	2 (2.7)	2 (3.1)	0 (0)	1

Table 136. Inflammatory profiles in patients with likely acute asthma compared to those with likely poorly controlled asthma.

Inflammatory profile	N= 74 (100%)	LAARA (n= 64, 86.5%)	LOCA (n= 10, 13.5%)	P value
	N (%) unless stated otherwise			
BEC Median (IQR) (n=72)	0.16 (0.06 – 0.45)	0.16 (0.05 – 0.43)	0.34 (0.15 – 0.48)	0.41
FeNO Median (IQR) (n=71)	29.0 (13.5 – 62.5)	26 (12 – 60)	44 (29.5 – 78.0)	0.06
SEC% Median (IQR) (n=42)	0.25 (0 – 4.1)	0.25 (0 – 4.4)	0.25 (0.13 – 2.0)	0.7
SNC% Median (IQR) (n=42)	91.3 (66.3 – 97.3)	88.5 (60.9 – 97.3)	96.0 (94.0 – 98.4)	0.09
SNC ≥65%	32 of 42 (76.2)	25 of 35 (71.4)	7 of 7 (100)	0.17
Raised $T2^{APEX3}$	47 (63.5)	38 (59.4)	9 (90.0)	0.08
• BEC ≥0.4 $x10^9$/L	20 of 72 (27.8)	17 of 63 (26.9)	3 of 9 (33.3)	0.70
• FeNO ≥25 ppb	40 of 71 (56.3)	31 of 61 (50.8)	9 of 9 (90.0)	**0.04**
• SEC ≥3%	14 of 42 (33.3)	12 of 35 (34.3)	2 of 7 (28.6)	1
Raised $T2^{APEX3}$/Neutrophils	60 (81.1)	50 (78.1)	10 (100)	0.19
Normal $T2^{APEX3}$/Neutrophils	14 (18.9)	14 (21.9)	0 (0)	
Raised $T2^{GINA}$	58 (78.4)	48 (75.0)	10 (100)	0.11
• BEC ≥0.15 $x10^9$/L	38 of 72 (52.8)	32 of 63 (50.8)	6 of 9 (66.7)	0.49
• FeNO ≥20 ppb	46 of 71 (64.8)	36 of 61 (59.0)	10 of 10 (100)	0.11
• SEC ≥2%	16 of 42 (38.1)	14 of 35 (40.0)	2 of 7 (28.6)	0.69
Raised $T2^{GINA}$/Neutrophils	67 (90.5)	57 (89.1)	10 (100)	0.58
Normal $T2^{GINA}$/Neutrophils	7 (9.5)	7 (10.9)	0 (0)	

BEC: Blood eosinophil count. FeNO: fractional exhaled nitric oxide. SEC: sputum eosinophils count. SNC: sputum neutrophils count.

Table 137. Cytokines profiling in patients with likely acute asthma compared to those with likely poorly controlled asthma.

Cytokine profiles	N= 74 (100%)	LAARA (n= 64, 86.5%)	LOCA (n= 10, 13.5%)	P value
	Median (IQR)			
T2-high cytokines				
• IL-5 Median (IQR) (n= 27)	2.8 (1.6 – 5.8)	3.1 (1.7 – 5.7)	1.6 (1.5 – 6.1)	0.56
• IL-13 Median (IQR) (n= 47)	238.7 (120.3 – 360.9)	244.9 (149.4 – 367.6)	68.4 (49.7 – 146.6)	**0.01**
• IL-4 Median (IQR) (n= 59)	41.9 (32.9 – 57.2)	44.4 (34.1 – 57.5)	34.5 (26.7 – 49.9)	0.27
Epithelial derived cytokines				
• IL-33 Median (IQR) (n= 56)	75.7 (49.2 – 121.7)	75.7 (49.2 – 122.5)	74.7 (57.3 – 113.1)	0.79
• TSLP Median (IQR) (n= 59)	1.5 (1.1 – 2.4)	1.7 (1.1 – 2.2)	1.4 (1.1 – 3.9)	0.69
Th17 cytokines				
• IL-17 Median (IQR) (n= 59)	395.6 (259.9 – 535.5)	381.3 (248.8 – 521.8)	430.9 (335.9 – 1091.7)	0.35
Pro-inflammatory cytokines				
• IL-8 Median (IQR) (n= 50)	1490.1 (769.4 – 2756.1)	1459.4 (747.7 – 2628.3)	2233.7 (868.2 – 3695.7)	0.31
• IL-1β Median (IQR) (n= 59)	52.6 (21.3 – 126.2)	46.6 (20.8 – 115.9)	82.2 (46.9 – 169.8)	0.48
• IL-6 Median (IQR) (n= 59)	21.3 (8.1 – 43.2)	20.7 (8.5 – 46.2)	24.0 (8.9 – 34.9)	0.91
• TNF-α Median (IQR) (n= 59)	14.1 (7.4 – 31.8)	13.6 (7.5 – 31.6)	21.9 (8.6 – 32.7)	0.54
Interferons				
• IFN-α Median (IQR) (n= 59)	10.4 (6.8 – 20.5)	10.4 (6.7 – 18.2)	10.6 (6.9 – 26.9)	0.72
• IFN-γ Median (IQR) (n= 58)	4.8 (2.9 – 8.3)	4.8 (3.2 – 7.4)	5.9 (2.6 – 13.2)	0.92
• IFN-β Median (IQR) (n= 35)	12.9 (3.5 – 20.3)	12.9 (3.9 – 19.0)	16.7 (2.0 – 35.7)	1

Table 138. Evidence of infection assessed on admission in patients with likely acute asthma compared to those with likely poorly controlled asthma.

Infection status	N= 74 (100%)	LAARA (n= 64, 86.5%)	LOCA (n= 10, 13.5%)	P value
	N (%)			
Positive sputum MC&S	5 of 41 (12.2)	4 of 34 (11.8)	1 of 7 (14.3)	
Positive PCR throat swab	9 of 34 (26.5)	9 of 30 (30.0)	0 of 4 (0)	0.55
CRP (>10 mg/L)	27 Of 73 (36.9)	25 of 64 (39.1)	2 of 9 (22.2)	0.47
BNC (>8 x10^9/L)	25 of 72 (34.7)	21 of 63 (33.3)	4 of 9 (44.4)	0.71
Overall positive infection status*	43 (58.1)	37 (57.8)	6 (60.0)	1

*MC&S: Sputum microscopy culture sensitivities. CRP: C-reactive protein. BNC: Blood neutrophil count. *Positive sputum MC&S, PCR throat swab, or increased CRP or BNC.*

Table 139. Acute asthma quality of life questionnaire assessed on admission in patients with likely acute asthma compared to those with likely poorly controlled asthma.

AAQLQ score	N= 74 (100%)	LAARA (n= 64, 86.5%)	LOCA (n= 10, 13.5%)	P value
	Mean ± SD			
AAQLQ overall score (n=73)	3.9 ± 1.5	3.9 ± 1.6	4.2 ± 1.4	0.64
• *Symptoms score (Mean ± SD)*	4.0 ± 1.5	3.9 ± 1.6	4.5 ± 1.2	0.24
• *Emotional score (Mean ± SD)*	3.9 ± 1.7	3.9 ± 1.7	3.8 ± 1.7	0.82

Table 140. Extrapulmonary comorbidity questionnaires assessed on admissions in patients with likely acute asthma compared to those with likely poorly controlled asthma.

Extra-pulmonary comorbidity questionnaires	N= 74 (100%)	LAARA (n= 64, 86.5%)	LOCA (n= 10, 13.5%)	P value
	N (%) unless stated otherwise			
SNOT-22 score Median (IQR) (n=47)	40.0 (25.5 – 60.0)	44.0 (24.3 – 62.5)	32.0 (29.0 – 33.5)	0.38
• Mild (8-20)	8 of 45 (17.8)	8 of 42 (19.1)	0 of 3 (0)	0.28
• Moderate (>20-50)	20 of 45 (44.4)	17 of 42 (40.5)	3 of 3 (100)	
• Severe (>50)	17 of 45 (37.8)	17 of 42 (40.5)	0 of 3 (0)	
• SNOT-22 >8 or Rhinitis diagnosis	71 (95.9)	61 (95.3)	10 (100)	1
GERDQ score (Mean ± SD) (n=56)	6.1 ± 2.3	6.1 ± 2.2	5.6 ± 2.8	0.64
• GERDQ >8	6 of 56 (10.7)	5 of 48 (10.4)	1 of 8 (12.5)	1
• GERDQ >8 or GERD diagnosis	23 (31.1)	20 (31.3)	3 (30.0)	1
PVCDI score (Mean ± SD) (n=70)	3.5 ± 2.8	3.8 ± 2.8	2.3 ± 2.1	0.07
• PVCDI score >3	32 of 70 (45.7)	29 of 60 (48.3)	3 of 10 (30)	0.32
• PVCDI score >3 or VCD diagnosis	34 (45.9)	31 (48.4)	3 (30)	0.32
Nijmegen score (Mean ± SD) (n=72)	21.9 ± 12.9	22.1 ± 13.3	21.3 ± 10.7	0.84
• Nijmegen Index >23	31 of 72 (43.1)	26 of 62 (41.9)	5 of 10 (50)	0.74
• Nijmegen Index >23 or Dysfunctional breathing diagnosis	31 (41.9)	26 (40.6)	5 (50)	0.73
HADS anxiety score Median (IQR) (n=46)	4.0 (2.0 – 6.8)	4 (2 – 7.3)	3.0 (2.0 – 5.5)	0.55
• HADS anxiety score >11	6 of 46 (13.0)	6 of 40 (15.0)	0 of 6 (0)	0.58
• HADS anxiety score >11 or anxiety diagnosis	34 (45.9)	30 (46.9)	4 (40)	0.75
HADS depression score Median (IQR) (n=42)	2.5 (1.0 – 5.0)	3 (0 – 5.3)	1.5 (1.0 – 2.8)	0.71
• HADS depression score >11	0 of 42 (0)	0 of 36 (0)	0 of 6 (0)	
• HADS depression score >11 or depression diagnosis	32 (43.2)	28 (43.8)	4 (40)	1

SNOT-22: Sino-nasal outcome test-22 questionnaire. GERDQ: Gastroesophageal reflux disease questionnaire. PVCDI: Pittsburgh Vocal Cord Dysfunction Index. HADS: Hospital Anxiety and Depression Scale.

Table 141. Body mass index and vitamin D in patients with likely acute asthma compared to those with likely poorly controlled asthma.

Other measures	N= 74 (100%)	LAARA (n= 64, 86.5%)	LOCA (n= 10, 13.5%)	P value
	N (%) unless stated otherwise			
BMI (Mean ± SD)	30.6 ± 8.6	30.3 ± 8.9	32.6 ± 6.1	0.31
BMI ranges				
• *Underweight (<18.5)*	1 (1.4)	1 (1.6)	0 (0)	0.17
• *Healthy range (18.5 – 24.9)*	19 (25.7)	19 (29.7)	0 (0)	
• *Overweight (25 – 29.9)*	19 (25.7)	16 (25.0)	3 (30)	
• *Obesity (30 – 39.9)*	25 (33.8)	19 (29.7)	6 (60)	
• *Severe obesity (≥40)*	10 (13.5)	9 (14.1)	1 (10)	
BMI ≥25 (overweight)	54 (72.9)	44 (68.8)	10 (100)	0.05
BMI ≥30 (obesity)	35 (47.3)	28 (43.8)	7 (70)	0.12
Vitamin D (Mean ± SD) (n=72)	49.8 ± 27.7	48.7 ± 28.1	56.6 ± 25.4	0.39
• *Vitamin D insufficiency (25-50 nmol/L)*	24 of 72 (33.3)	23 of 62 (37.1)	1 of 10 (10)	0.14
• *Vitamin D deficiency (<25 nmol/L)*	15 of 72 (20.8)	13 of 62 (20.9)	2 of 10 (20)	1

Table 142. Assessment of adherence in patients with likely acute asthma compared to those with likely poorly controlled asthma.

Previously diagnosed asthma	N= 65	LAARA (n= 55)	LOCA (n= 10)	P value
Adherence	N (%) unless stated otherwise			
MARS (n= 63) Median (IQR)	23 (19 – 25)	23 (20 – 25)	23 (19 – 24.8)	1
MPR (n= 59) Median (IQR)	50 (8.3 – 100)	50 (8.3 – 100)	57 (12 – 102.5)	0.71
MPR <75%	33 of 59 (55.9)	29 of 51 (56.9)	4 of 8 (50)	0.72
On GINA treatment Step	52 (80)	44 (80)	8 (80)	1
Inhaler technique (n= 49)				
• *Poor*	6 (12.2)	6 (14.3)	0 (0)	0.86
• *Good*	21 (42.9)	18 (42.9)	3 (42.9)	
• *Excellent*	22 (44.9)	18 (42.9)	4 (57.1)	
• *Total*	49	42	7	

MARS: Medication adherence report scale questionnaire. MPR: Medication Possession Ratio.

Appendix I: APEX3- Lung function and inflammation in patients with high body mass index (≥30, HBMI, n= 40) vs. low body mass index (<30, LBMI, n= 43)

Summary of findings

Lung function measures

Both mean FEV_1% and FEV_1/FVC were significantly lower in patients with LBMI compared to those with HBMI (Table 143). Mean R5 value was not different between the groups (Table 144). Patients with LBMI tended to be likely to have evidence of airflow obstruction as determined by FEV_1/FVC and/or R5.

Inflammatory profile

The median FeNO value and the proportion of those with FeNO ≥25 ppb were both higher in the LBMI compared to the HBMI group (Table 146). The number of patients with raised $T2^{APEX3}$ markers tended to be higher in the LBMI group (Table 146).

Table 143. Spirometry parameters in patients with high BMI compared to those with low BMI.

Spirometry parameters	HBMI (n= 40, 48.2%)	LBMI (n= 43, 51.8%)	P value
	N (%) unless stated otherwise		
On admission FEV_1% (Mean ± SD)	71.9 ± 21.1	60.1 ± 22.1	**0.01**
On admission FVC% (Mean ± SD)	79.8 ± 19.3	78.4 ± 19.1	0.74
On admission FEV_1/FVC (Mean ± SD)	73.2 ± 9.3	62.3 ± 15.4	**<0.001**
On admission PEFR% (Mean ± SD)	84.4 ± 19	74.9 ± 26.6	0.07
FEV_1 <80%	27 (67.5)	33 (76.7)	0.35
FVC <80%	21 (52.5)	23 (53.5)	0.92
FEV_1 >80% and FVC <80%	1 (2.5)	0 (0)	0.48
FEV_1/FVC <70%	18 (45.0)	30 (69.8)	**0.02**
PEFR <75%	13 (32.5)	20 (46.5)	0.19

Table 144. Forced oscillation technique parameters in patients with high BMI compared to those with low BMI.

FOT parameters	HBMI (n= 40, 48.2%)	LBMI (n= 43, 51.8%)	P value
	N (%) unless stated otherwise		
On admission R5 (Mean ± SD)	5.7 ± 2.1	5.5 ± 2.6	0.76
R5 >5	22 (55.9)	21 (48.8)	0.57

Table 145. Small airway involvement in patients with high BMI compared to those with low BMI.

Small airway disease	HBMI (n= 40, 48.2%)	LBMI (n= 43, 51.8%)	P value
	N (%) unless stated otherwise		
On admission $FEF_{25-75\%}$ (Mean ± SD)	57.4 ± 28.7	39.4 ± 27.8	**0.004**
On admission R5-R20 (Mean ± SD)	1.7 ± 1.4	1.8 ± 1.8	0.79
$FEF_{25-75\%}$ <65%	22 (55.0)	33 (76.7)	**0.03**
R5-R20 >1	25 (62.5)	24 (55.8)	0.53
Small airway disease*	2 (5)	6 (13.9)	0.27

**Defined as per Lipworth's criteria: Preserved FEV_1 (>80%) and (FEF_{25-75} <60% OR R5-20 >1 $cmH_2O/L/s$).*

Table 146. Inflammatory profiles in patients with high BMI compared to those with low BMI.

Inflammatory profile	HBMI (n= 40, 48.2%)	LBMI (n= 43, 51.8%)	P value
	N (%) unless stated otherwise		
BEC Median (IQR) (n= 81)	0.16 (0.08 – 0.38)	0.13 (0.03 – 0.41)	0.35
FeNO Median (IQR) (n= 80)	19.5 (9.3 – 37.8)	39.0 (14.0 – 78.3)	**0.02**
SEC% Median (IQR) (n= 45)	0.25 (0.0 – 1.5)	1.1 (0.0 – 6.8)	0.19
SNC% Median (IQR) (n= 45)	89.8 (59.8 – 97.3)	88.0 (60.9 – 97.3)	1
SNC ≥65%	15 of 21 (71.4)	17 of 24 (70.8)	0.9
Raised $T2^{APEX3}$	19 (47.5)	28 (65.1)	0.11
• BEC ≥0.4 $x10^9$/L	9 of 38 (23.7)	11 of 43 (25.6)	0.84
• FeNO ≥*25* ppb	14 of 38 (36.8)	26 of 42 (61.9)	**0.02**
• SEC ≥3%	5 of 21 (23.8)	9 of 24 (37.5)	0.32
Raised $T2^{APEX3}$ /Neutrophils	27 (67.5)	33 (76.7)	0.34
Normal $T2^{APEX3}$ /Neutrophils	13 (32.5)	10 (23.2)	

BEC: Blood eosinophil count. FeNO: fractional exhaled nitric oxide. SEC: sputum eosinophils count. SNC: sputum neutrophils count.

Appendix J: APEX3- Lung function, inflammation, asthma treatment, and medication prescription rate in frequent exacerbators (>1 OCS in the last 12 months, FE, n= 37) vs. less frequent exacerbators (≤1 OCS in the last 12 months, LFE, n= 46)

Summary of findings

Lung function measures

Mean FEV_1%, FVC%, and lung function ratio were similar between the groups (Table 147). The number of patients with impaired FEV_1/FVC tended to be greater in the LFE compared to the FE (Table 147). Mean PEFR% was lower, and the proportion of those with PEFR <75% was higher, in the LFE compared to the FE (Table 147).

Inflammatory profile

Both median BEC and FeNO were higher in the LFE compared to the FE (Table 150). The proportion of patients with raised $T2^{APEX3}$ markers was higher in the LFE compared to the FE (Table 150). The proportion of those who had OCS treatment prior to hospital admission was higher in the FE compared to the LFE.

Asthma treatment and medication prescription rate

Frequent exacerbators were more likely to have previously diagnosed asthma and to be on asthma medications at the time of study recruitment (Table 151). The proportion of patients on GINA step 5 treatment step and biological treatment or low-dose maintenance OCS was significantly higher in the FE compared to the LFE (Table 151). Median medication prescription rate was higher, and the proportion of those with MPR <75% was lower in the FE compared to the LFE (Table 151).

Table 147. Spirometry parameters in frequent vs. less frequent exacerbators.

Spirometry parameters	FE (n= 37, 44.6%)	LFE (n= 46, 55.4%)	P value
	N (%) unless stated otherwise		
On admission FEV_1% (Mean ± SD)	68.9 ± 22.6	63.4 ± 21.9	0.26
On admission FVC% (Mean ± SD)	80.8 ± 19.2	77.6 ± 19.1	0.44
On admission FEV_1/FVC (Mean ± SD)	68.6 ± 14.4	66.7 ± 13.5	0.54
On admission PEFR% (Mean ± SD)	86.7 ± 25.5	73.7 ± 20.5	**0.01**
FEV_1 <80%	26 (70.3)	34 (73.9)	0.72
FVC <80%	16 (43.2)	28 (60.9)	0.10
FEV_1 >80% and FVC <80%	1 (2.7)	0 (0)	0.44
FEV_1/FVC <70%	18 (48.7)	30 (65.2)	0.12
PEFR <75%	6 (20.7)	27 (50.0)	**0.002**

Table 148. Forced oscillation technique parameters in frequent vs. less frequent exacerbators.

FOT parameters	FE (n= 37, 44.6%)	LFE (n= 46, 55.4%)	P value
	N (%) unless stated otherwise		
On admission R5 (Mean ± SD)	5.8 ± 2.9	5.4 ± 1.9	0.46
R5 >5	18 (48.7)	25 (54.4)	0.61

Table 149. Small airway involvement in frequent vs. less frequent exacerbators.

Small airway disease	FE (n= 37, 44.6%)	LFE (n= 46, 55.4%)	P value
	N (%) unless stated otherwise		
On admission $FEF_{25-75\%}$ (Mean ± SD)	54.1 ± 29.2	43.2 ± 29.1	0.09
On admission R5-R20 (Mean ± SD)	1.8 ± 1.9	1.7 ± 1.3	0.68
$FEF_{25-75\%}$ <65%	20 (54.1)	35 (76.1)	**0.03**
R5-R20 >1	21 (56.8)	28 (60.9)	0.70
Small airway disease*	3 (8.11)	5 (10.9)	0.48

**Defined as per Lipworth's criteria: Preserved FEV_1 (>80%) and (FEF_{25-75} <60% OR R5-20 >1 $cmH_2O/L/s$).*

Table 150. Inflammatory profiles in frequent vs. less frequent exacerbators.

Inflammatory profile	FE (n= 37, 44.6%)	LFE (n= 46, 55.4%)	P value
	N (%) unless stated otherwise		
Pre-admission OCS	**12 (32.4)**	**4 (8.7)**	**0.006**
BEC Median (IQR) (n= 81)	0.06 (0.01 – 0.23)	0.30 (0.10 – 0.49)	**<0.001**
FeNO Median (IQR) (n= 80)	20.0 (9.5 – 33.3)	39.0 (14.3 – 66.8)	0.06
SEC% Median (IQR) (n= 45)	0.25 (0.0 – 1.8)	0.5 (0.0 – 6.9)	0.21
SNC% Median (IQR) (n= 45)	95.8 (73.6 – 97.3)	87.5 (52.5 – 96.3)	0.17
SNC ≥65%	16 of 20 (80.0)	16 of 25 (64.0)	0.32
Raised $T2^{APEX3}$	13 (35.1)	34 (73.9)	**<0.001**
• BEC ≥0.4 $x10^9$/L	3 of 35 (8.6)	17 of 46 (36.9)	**0.01**
• FeNO ≥25 ppb	13 of 36 (36.1)	27 of 44 (61.4)	**0.02**
• SEC ≥3%	3 of 20 (15.0)	11 of 25 (44.0)	0.05
Raised $T2^{APEX3}$/Neutrophils	22 (59.5)	38 (82.6)	**0.01**
Normal $T2^{APEX3}$/Neutrophils	15 (40.5)	8 (17.4)	

BEC: Blood eosinophil count. FeNO: fractional exhaled nitric oxide. SEC: sputum eosinophils count. SNC: sputum neutrophils count.

Table 151. Asthma treatment and MPR in frequent vs. less frequent exacerbators.

Previous asthma diagnosis	FE (n= 37)	LFE (n= 37)	P value
Asthma treatment and MPR	N (%) unless stated otherwise		
Not on asthma treatment	0 (0)	9 (24.3)	**0.001**
SABA only	2 (5.4)	2 (5.4)	1
On GINA treatment Step	35 (94.6)	26 (70.3)	**0.005**
• *Step 1*	1 (2.9)	0 (0)	**<0.001**
• *Step 2*	0 (0)	4 (15.4)	
• *Step 3*	4 (11.4)	6 (23.1)	
• *Step 4*	12 (34.3)	15 (57.7)	
• *Step 5*	18 (51.4)	1 (3.9)	
Biologics	3 (8.1)	0 (0)	0.23
Low-dose maintenance OCS	2 (5.4)	0 (0)	0.49
Biologics or Low-dose maintenance OCS	5 (13.5)	0 (0)	0.05
MPR Median (IQR) (n= 68)	100 (58.3 – 100)	16 (0 – 56.0)	**<0.001**
MPR <75%	10 (29.4)	26 (76.4)	**<0.001**

**In those with previous asthma diagnosis.*

Appendix K: APEX3- Paired visits in all patients who completed V2 (n=60)

Summary of findings

A total of 60 patients completed V2, either attended in-person (57, 95.0%) or as a phone call follow-up (3, 5.0%). 37 of 97 (38.1%) patients missed visit 2, and 50 of 97 (51.5%) missed V3.

Demographics of patients at V2

The majority of the patients were white and female with a mean age of 45.7 years. Demographic characteristics of patients at V1 were largely similar to those of patients at V2 (Table 152).

Lung function parameters

Of those who completed V2 (n= 60), 57 (95%) attended in person, of whom 47 (82.5%) were able to perform spirometry at both visits. In patients with paired spirometry measurements (n= 47), FEV_1% and FEV_1/FVC were significantly higher at V2 compared to V1 (Table 153). Of those who completed V2 (n= 60), 57 (95%) attended in person, of whom 56 (98.2%) had paired FOT measurements. The mean R5 value was significantly greater at V2 compared to V1 (Table 154).

Of those who completed both measures of airflow obstruction at both visits (n= 47), 21 (44.7%) had normal spirometry and FOT at V2. 26 (55.3%) had evidence of airflow obstruction at V2 on either or both measures compared to 37 (78.7%) at V1. Of those with airflow limitation at V2 (n= 26), 9 (19.2%) had normal spirometry and obstructive FOT, 10 (21.3%) had normal FOT and obstructive spirometry, and 7 (14.9%) had evidence of airflow limitation on both measures (Figure 40). $FEF_{25-75\%}$ was significantly higher, and R5-20 was significantly lower at V2 compared to V1 (Table 155).

Inflammatory profile

Paired blood differential count data are available for 55 patients. There was no difference in the paired median BEC value between visits (Table 156). There was a significant shift towards a normal BEC at V2 compared to V1 (Figure 41). 55 patients had successfully completed FeNO at both visits. FeNO was significantly lower at V2

compared to V1. The proportion of patients with increased FeNO was not different between visits (Figure 42). 10 paired sputum samples are available. The median sputum eosinophil percentage was significantly lower at V2 compared to V1. The mean sputum neutrophils count was higher at V2 compared to V1 (Table 156).

32 (56.1%) of the patients had evidence of raised $T2^{APEX3}$ inflammatory markers at V2 compared to 34 (59.7%) at V1 (Table 156) (Figure 43). 6 of 23 (26.1%) patients with normal markers of T2 inflammation at V1 had increased markers of T2 inflammation at V2.

T2-high, epithelial-derived, Th-17 cytokines, and interferons were all similar at V2 compared to V1 (Table 157). Pro-inflammatory cytokines were also not different between visits except for IL-6, which was significantly higher at V1 compared to V2 (Table 157).

Evidence of infection

The proportion of patients with increased c-reactive protein was higher at V1 compared to V2 (Table 158). Similarly, the number of patients with increased blood neutrophil count was higher at V1 compared to V2 (Table 158).

Symptoms score

The overall AAQLQ score was significantly higher at V2 compared to V1 (Table 159). The symptoms and emotional domains of the questionnaire were all significantly higher at V2 compared to V1. The overall score, symptoms score, and emotional score of the AAQLQ were all significantly higher at V3 compared to V1 but remained largely unchanged from V2 to V3 (Table 160).

Table 152. Demographic characteristics of patients at V2.

Demographics	V1: N= 97	V2: N= 60
	N (%) unless otherwise stated	
Age (Mean ± SD) (years)	43.9 ± 17.0	45.7 ± 16.7
Gender		
• *Male*	32 (33.0)	18 (30.0)
• *Female*	65 (67.0)	42 (70.0)
Ethnicity		
• *White/Caucasian*	69 (71.1)	44 (73.3)
• *Black/Black British*	10 (10.3)	6 (10.0)
• *Asian/Asian British*	13 (13.4)	6 (10.0)
• *Other*	5 (5.2)	4 (6.7)

Table 153. Spirometry parameters at V1 compared to V2.

Spirometry parameters	V1 (n= 47)	V2 (n= 47)	P value
	N (%) unless otherwise stated		
FEV_1% (Mean ± SD)	66.0 ± 21.9	83.7 ± 16.5	**<0.001**
FVC% (Mean ± SD)	80.5 ± 17.1	93.2 ± 14.5	**<0.001**
FEV_1/FVC (Mean ± SD)	65.9 ± 15.1	73.3 ± 11.3	**<0.001**
PEFR% (Mean ± SD)	80.6 ± 24.3	100.5 ± 18.1	**<0.001**
FEV_1 <80%	31 (65.9)	19 (40.4)	0.69
FVC <80%	23 (48.9)	8 (17.0)	**0.04**
FEV_1 >80% and FVC <80%	1 (2.1)	0 (0)	**<0.001**
FEV_1/FVC <70%	28 (59.6)	17 (36.2)	0.79
PEFR <75%†	17 (36.2)	3 (6.4)	**0.005**

†Compared to best PEFR value or predicted PEFR if best is not available.

Table 154. Forced oscillation technique measures at V1 compared to V2.

FOT measures	V1 (n= 56)	V2 (n= 56)	P value
	N (%) unless otherwise stated		
R5 (Mean ± SD)	5.4 ± 2.3	4.5 ± 1.7	**<0.001**
R5 >5	28 (50.0)	17 (30.4)	0.17

Figure 40. Evidence of airflow limitation at V2.

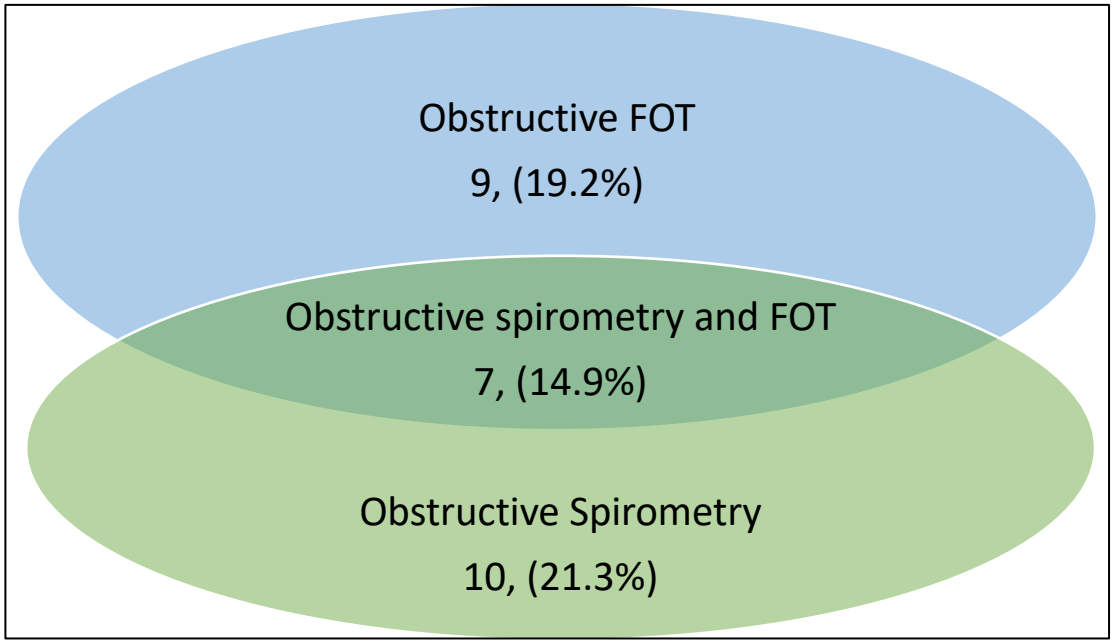


Table 155. Small airway measures at V1 compared to V2.

Small airway disease	V1	V2	P value
	N (%) unless otherwise stated		
$FEF_{25-75\%}$ (Mean ± SD) (n= 47)	48.1 ± 29.4	67.9 ± 26.5	**<0.001**
R5-R20 (Mean ± SD) (n= 56)	1.7 ± 1.5	1.1 ± 0.9	**0.001**
$FEF_{25-75\%}$ <65%	30 of 47 (63.8)	18 of 47 (38.3)	0.89
R5-R20 >1	37 of 56 (66.1)	25 of 56 (44.6)	0.46
Small airway disease†	7 of 47 (14.9)	13 of 47 (27.7)	**<0.001**

†Defined as per Lipworth's criteria: Preserved FEV_1 (>80%) and (FEF_{25-75} <60% OR R5-20 >1 CmH2O/L/s).

Table 156. Inflammatory biomarkers at V1 compared to V2.

Inflammatory measures	V1	V2	P value
	N (%) unless otherwise stated		
BEC Median (IQR) (n= 55)	0.16 (0.0- 0.4)	0.20 (0.1- 0.3)	0.62
FeNO Median (IQR) (n= 55)	37 (12.5- 73.5)	28 (12- 51.5)	**0.03**
SEC% Median (IQR) (n= 10)	11.8 (0.7- 24.6)	0.8 (0.1- 3.1)	**0.04**
SNC% (Mean ± SD) (n= 10)	62.2 ± 18.5	86.7 ± 14.8	**0.02**
SNC ≥65%	5 of 10 (50)	8 of 10 (80)	0.44
Raised T2^{APEX3}	34 of 57 (59.7)	32 of 57 (56.1)	0.24
• BEC ≥0.4 x10^9/L	14 of 55 (25.5)	10 of 55 (18.2)	**<0.001**
• FeNO ≥25 ppb	31 of 55 (56.4)	29 of 55 (52.7)	0.51
• SEC ≥3%	6 of 10 (60)	3 of 10 (30)	NA
Raised T2^{GINA}	44 of 57 (77.2)	42 of 57 (73.7)	**<0.001**
• BEC ≥0.15 x10^9/L	30 of 55 (54.6)	35 of 55 (63.6)	0.15
• FeNO ≥20 ppb	34 of 55 (61.8)	32 of 55 (58.2)	0.14
• SEC ≥2%	7 of 10 (70)	3 of 10 (30)	NA

Figure 41. Changes in BEC at V2 compared to V1.

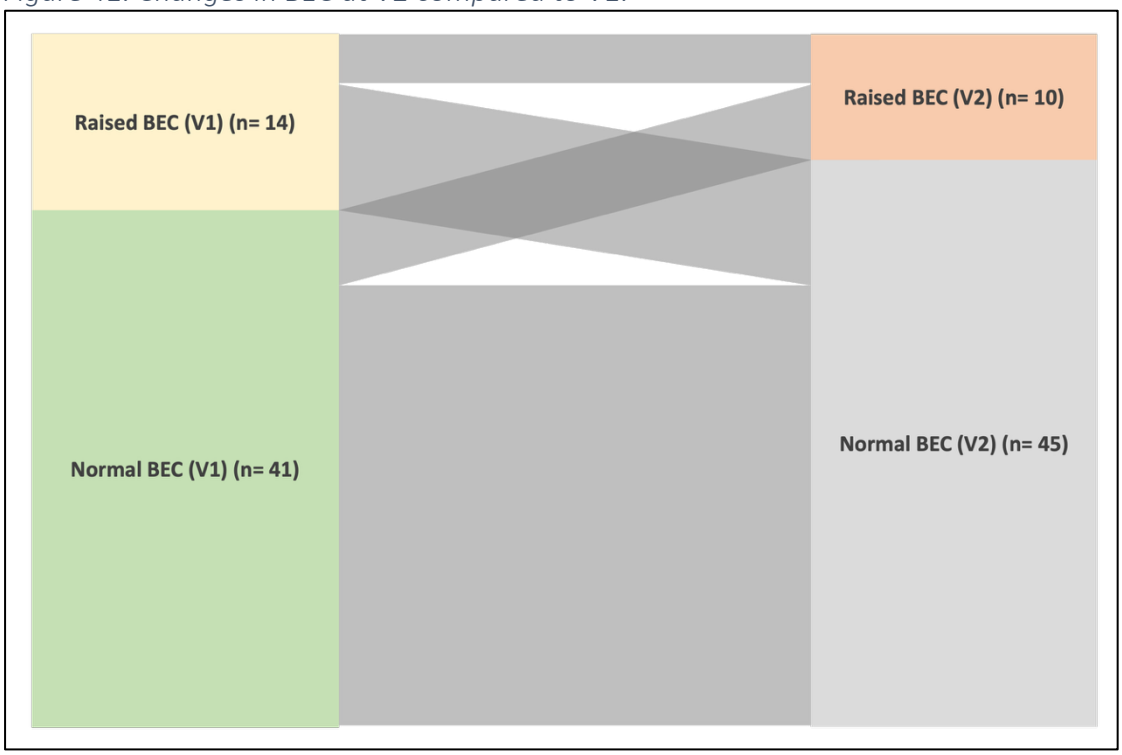


Figure 42. Changes in FeNO at V2 compared to V1.

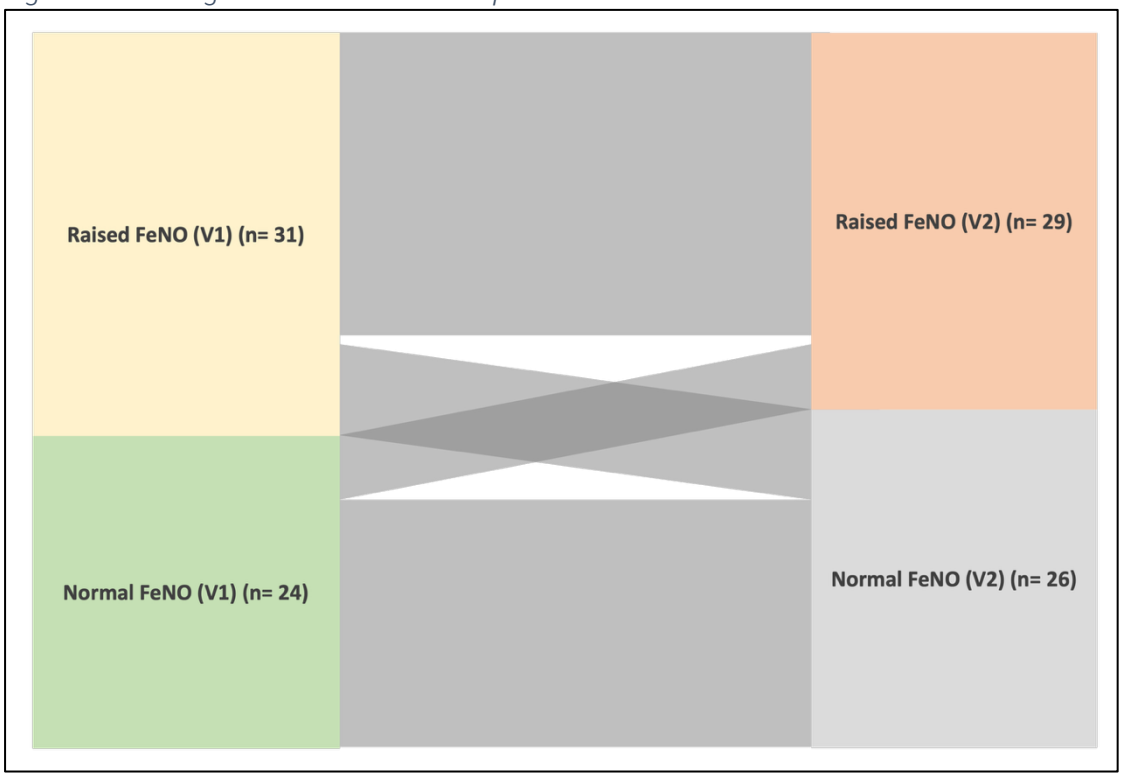


Figure 43. Changes in T2-high inflammation at V2 compared to V1.

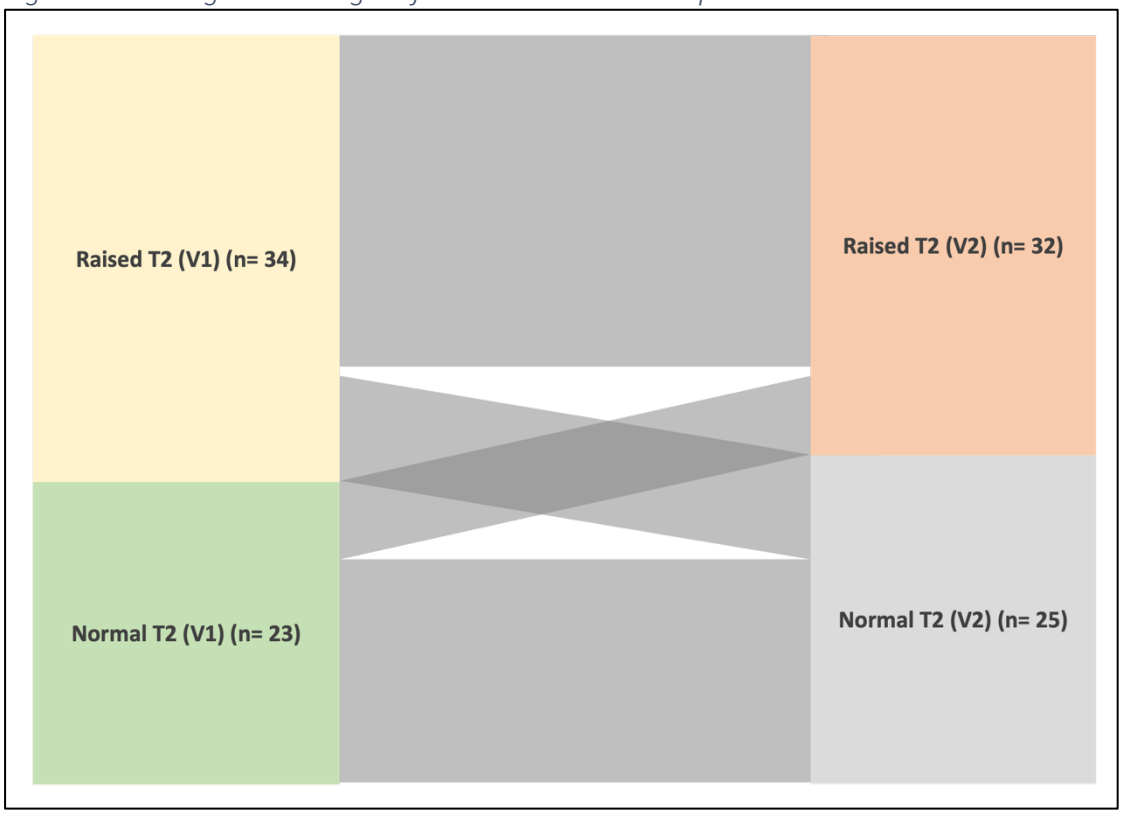


Table 157. Cytokines profiling at V2 compared to V1.

Cytokine profiles	V1	V2	P value
T2-high cytokines	Median (IQR)		
• IL-5 Median (IQR) (n= 12)	1.8 (1.5 – 3.4)	3.2 (1.8 – 6.1)	0.45
• IL-13 Median (IQR) (n= 31)	244.9 (104.7 – 417.7)	375.5 (212.9 – 542.1)	0.39
• IL-4 Median (IQR) (n= 41)	41.9 (31.9 – 61.2)	46.3 (28.6 – 71.2)	0.81
Epithelial derived cytokines			
• IL-33 Median (IQR) (n= 35)	73.2 (46.4 – 131.3)	62.0 (34.2 – 90.8)	0.09
• TSLP Median (IQR) (n= 41)	1.7 (1.1 – 2.2)	1.7 (0.9 – 2.6)	0.78
Th17 cytokines			
• IL-17 Median (IQR) (n= 41)	395.6 (278.5 – 486.5)	404.1 (336.5 – 743.0)	0.36
Pro-inflammatory cytokines			
IL-8 Median (IQR) (n= 35)	1502.9 (541.5 –2750.8)	1035.2 (705.9 – 2764.3)	0.93
• IL-1β Median (IQR) (n= 41)	47.6 (26.2 – 115.9)	63.3 (36.6 – 109.2)	0.73
• IL-6 Median (IQR) (n= 41)	14.2 (7.6 – 34.6)	7.2 (3.5 – 15.9)	**0.002**
• TNF-α Median (IQR) (n= 41)	14.2 (7.3 – 24.2)	16.9 (13.0 – 23.9)	0.95
Interferons			
• IFN-α Median (IQR) (n= 41)	11.3 (6.7 – 21.6)	14.4 (8.6 – 22.7)	0.95
• IFN-γ Median (IQR) (n= 41)	4.9 (2.9 – 8.4)	5.2 (2.9 – 7.9)	0.73
• IFN-β Median (IQR) (n= 20)	12.3 (3.6 – 20.1)	13.3 (10.4 – 24.6)	0.17

Table 158. Measures of infection at V1 compared to V2.

Infection status	V1	V2	P value
	N (%)		
CRP (> 10 mg/L)	20 of 57 (35.1)	9 of 57 (15.8)	**<0.001**
BNC (> 8 x10^9/L)	17 of 55 (30.9)	13 of 55 (23.6)	**0.001**

CRP: c-reactive protein. BNC: blood neutrophil count.

Table 159. Acute Asthma Quality of Life Questionnaire at V1 compared to V2.

AAQLQ	V1 (n= 60)	V2 (n= 60)	P value
	N (%) unless otherwise stated		
AAQLQ overall score Median (IQR)	3.6 (3.0- 5.3)	6.4 (4.9- 6.7)	**<0.001**
Minimum important difference			
• *No difference between visits (<0.5)*	13 (21.7)		
• *Minimum difference (≥0.5)*	10 (16.7)		
• *Moderate difference (>1.0)*	5 (8.3)		
• *Large difference (>1.51)*	32 (53.3)		
Symptoms score Median (IQR)	3.7 (2.8- 5.8)	6.3 (5.3- 6.8)	**<0.001**
Minimum important difference			
• *No different between visits (<0.5)*	13 (21.7)		
• *Minimum difference (≥0.5)*	6 (10.0)		
• *Moderate difference (>1.0)*	10 (16.7)		
• *Large difference (>1.51)*	31 (51.7)		
Emotional score Median (IQR)	3.8 (2.6- 5.4)	6.2 (4.6- 6.7)	**<0.001**
Minimum important difference			
• *No different between visits (<0.5)*	19 (31.7)		
• *Minimum difference (≥0.5)*	5 (8.3)		
• *Moderate difference (>1.0)*	9 (15.0)		
• *Large difference (>1.51)*	27 (45.0)		

Table 160. Acute Asthma Quality of Life Questionnaire at V3 compared to V2 and V1.

AAQLQ	V1 (n= 47)	V3 (n= 47)	P value	V2 (n= 47)	V3 (n= 47)	P value
	Median (IQR)			Median (IQR)		
Overall score†	3.5 (2.9- 5.1)	6.4 (5.4- 6.7)	**<0.001**	6.5 (5.0- 6.7)	6.4 (5.4- 6.7)	0.75
Symptoms score†	3.67 (2.7- 5.0)	6.3 (5.8 – 7.0)	**<0.001**	6.3 (5.3- 6.8)	6.3 (5.8- 7.0)	0.96
Emotional score†	3.4 (2.4- 5.0)	6.4 (4.9- 6.7)	**<0.001**	6.2 (4.6- 6.6)	6.4 (4.9- 6.7)	0.56

†In those who completed V3.

Appendix L: APEX3- Analysis of follow-up visits-summary of missing data.

Paired visits in all patients who completed V2 (n=60) -summary of missing data.

Table 161. Demographic characteristics of patients at V2-missing data.

Demographics	V2: N= 60
	N (%)
Age	0 (0)
Gender	0 (0)
Ethnicity	0 (0)

Table 162. Lung function measures of patients at V2-missing data.

Lung function measures	V2: N= 60
	N (%)
Spirometry	13 (21.7)
Forced oscillation	4 (6.7)

Table 163. Inflammatory measures of patients at V2-missing data.

Inflammatory measures	V2: N= 60
	N (%)
Blood eosinophil count	5 (8.3%)
FeNO	5 (8.3%)
Sputum inflammatory markers	50 (83.3%)

Table 164. Cytokines profiling of patients at V2-missing data.

Cytokine profiles	V2: N= 60
T2-high cytokines	N (%)
• IL-5	48 (80.0)
• IL-13	29 (48.3)
• IL-4	19 (31.7)
Epithelial derived cytokines	
• IL-33	25 (41.7)
• TSLP	19 (31.7)
Th17 cytokines	
• IL-17	19 (31.7)
Pro-inflammatory cytokines	
IL-8	25 (41.7)
• IL-1β	19 (31.7)
• IL-6	19 (31.7)
• TNF-α	19 (31.7)
Interferons	
• IFN-α	19 (31.7)
• IFN-γ	19 (31.7)
• IFN-β	40 (66.7)

Table 165. Evidence of infection of patients at V2-missing data.

Infection measures	V2: N= 60
	N (%)
CRP	3 (5%)
BNC	5 (8.3%)

Table 166. Acute Asthma Quality of Life Questionnaire of patients at V2-missing data.

Acute Asthma Quality of Life Questionnaire	V2: N= 60
	N (%)
AAQLQ	0 (0)

Paired visits in the LAARA group (n= 41)-summary of missing data.

Table 167. Demographic characteristics of patients in the LAARA group at V2-missing data.

Demographics (V2)	n= 41
	N (%)
Age	0 (0)
Gender	0 (0)
Ethnicity	0 (0)

Table 168. Lung function measures of patients in the LAARA group at V2-missing data.

Lung function measures	n= 41
	N (%)
Spirometry	4 (9.8)
Forced oscillation	3 (7.3)

Table 169. Inflammatory measures of patients in the LAARA group at V2-missing data.

Inflammatory measures	n= 41
	N (%)
Blood eosinophil count	5 (12.2%)
FeNO	4 (9.8)
Sputum inflammatory markers	34 (82.9%)

Table 170. Cytokines profiling of patients in the LAARA group at V2-missing data.

Cytokine profiles	n= 41
T2-high cytokines	N (%)
• IL-5	35 (85.4)
• IL-13	23 (56.1)
• IL-4	17 (41.5)
Epithelial derived cytokines	
• IL-33	22 (53.7)
• TSLP	17 (41.5)
Th17 cytokines	
• IL-17	17 (41.5)
Pro-inflammatory cytokines	
IL-8	20 (48.8)
• IL-1β	17 (41.5)
• IL-6	17 (41.5)
• TNF-α	17 (41.5)
Interferons	
• IFN-α	17 (41.5)
• IFN-γ	17 (41.5)
• IFN-β	29 (70.7)

Table 171. Evidence of infection of patients in the LAARA group at V2-missing data.

Infection measures	n= 41
	N (%)
CRP	3 (7.3)
BNC	5 (12.2%)

Table 172. Acute Asthma Quality of Life Questionnaire of patients in the LAARA group at V2-missing data.

Acute Asthma Quality of Life Questionnaire	n= 41
	N (%)
AAQLQ	0 (0)

Paired visits in the LINAA group (n= 10)-summary of missing data.

Table 173. Demographic characteristics of patients in the LINAA group at V2-missing data.

Demographics (V2)	n= 10
	N (%)
Age	0 (0)
Gender	0 (0)
Ethnicity	0 (0)

Table 174. Lung function measures of patients in the LINAA group at V2-missing data.

Lung function measures	n= 10
	N (%)
Spirometry	0 (0)
Forced oscillation	0 (0)

Table 175. Inflammatory measures of patients in the LINAA group at V2-missing data.

Inflammatory measures	n= 10
	N (%)
Blood eosinophil count	0 (0)
FeNO	0 (0)
Sputum inflammatory markers	9 (90.0%)

Table 176. Cytokines profiling of patients in the LINAA group at V2-missing data.

Cytokine profiles	n= 10
T2-high cytokines	N (%)
• IL-5	7 (70.0)
• IL-13	5 (50.0)
• IL-4	1 (10.0)
Epithelial derived cytokines	
• IL-33	2 (20.0)
• TSLP	1 (10.0)
Th17 cytokines	
• IL-17	1 (10.0)
Pro-inflammatory cytokines	
IL-8	2 (20.0)
• IL-1β	1 (10.0)
• IL-6	1 (10.0)
• TNF-α	1 (10.0)
Interferons	
• IFN-α	1 (10.0)
• IFN-γ	1 (10.0)
• IFN-β	7 (70.0)

Table 177. Evidence of infection of patients in the LINAA group at V2-missing data.

Infection measures	n= 10
	N (%)
CRP	0 (0)
BNC	0 (0)

Table 178. Acute Asthma Quality of Life Questionnaire of patients in the LINAA group at V2-missing data.

Acute Asthma Quality of Life Questionnaire	n= 10
	N (%)
AAQLQ	0 (0)

Changes in lung function, inflammation, and symptom scores between visits in the Likely acute asthma-related admissions (LAARA, n=41) vs. Likely not acute-asthma admissions (LINAA, n=10)- summary of missing data.

Table 179. Changes in lung function parameters between visits in the LAARA compared to the LINAA-missing data.

Lung function measures	LAARA (n= 41)	LINAA (n= 10)
	N (%)	
Spirometry	4 (9.8)	0 (0)
Forced oscillation	3 (7.3)	0 (0)

Table 180. Changes in inflammatory biomarkers between visits in the LAARA compared to the LINAA-missing data.

Inflammatory biomarkers	LAARA (n= 41)	LINAA (n= 10)
	N (%)	
Blood eosinophil count	5 (12.2%)	0 (0)
FeNO	4 (9.8)	0 (0)
Sputum inflammatory markers	34 (82.9%)	9 (90.0%)

Table 181. Changes in symptoms scores between visits in the LAARA compared to the LINAA-missing data.

Acute Asthma Quality of Life Questionnaire	LAARA (n= 41)	LINAA (n= 10)
	N (%)	
AAQLQ	0 (0)	0 (0)

Changes in lung function and symptom scores between visits in patients with T2-low (n= 24) compared to T2-high (n= 36) asthma attacks as per APEX3 criteria for T2-high inflammation (FeNO level ≥25 ppb, blood eosinophil count of ≥0.4 x10^9/L, or sputum eosinophil count of ≥3%)- summary of missing data.

Table 182. Changes in lung function parameters between visits in patients with initially T2-high asthma attacks compared to T2-low asthma attacks-missing data.

Lung function measures	T2-low (n= 24)	T2-high (n= 36)
	N (%)	
Spirometry	4 (16.7)	9 (25.0)
Forced oscillation	2 (8.3)	2 (5.6)

Table 183. Changes in AAQLQ scores between visits in patients with initially T2-high asthma attacks compared to T2-low asthma attacks-missing data.

Acute Asthma Quality of Life Questionnaire	T2-low (n= 24)	T2-high (n= 36)
AAQLQ	0 (0)	0 (0)

Appendix M: APEX3- Paired visits in the LOCA group (n= 7)

Summary of findings

All the patients from the LOCA group attended V2. The median number of days between hospital discharge and V2 in the LOCA group is 13 days (IQR= 12 days – 14 days).

Demographics

The majority of the patients who attended V2 were white female with a median age of 34.9 years (Table 184).

Lung function parameters at V1 compared to V2 in the LOCA group

The median FEV_1% and the proportion of patients with impaired FEV_1% were similar between visits (Table 185). The median lung function ratio, however, was slightly reduced at V2 compared to V1 (Figure 44). Mean R5 value tended to be increased at V2 compared to V1 (Table 186) (Figure 45). Overall, 4 (57.1%) of the patients in the LOCA group had evidence of airflow limitation on either or both measures at V2. Mean $FEF_{25-75\%}$ was higher at V1 compared to V2 (Table 187).

Inflammatory profile at V2 compared to V1 in the LOCA group

The median BEC was similar between the visits (Table 188). FeNO tended to be higher at V1 compared to V2. The difference in sputum inflammatory markers was not assessed due to the limited number of paired samples obtained.

Evidence of infection

The proportion of patients with infection was similar between visits (Table 189).

Symptoms score

The AAQLQ scores tended to be slightly higher at V2 compared to V1 although this was not statistically significant (Table 190). Similarly, AAQLQ scores were not different at V3 compared to V1 (Table 191).

Table 184. Demographic characteristics of patients in the LOCA group at V2.

Demographics (V2)	n= 7
	N (%) unless stated otherwise
Age Median (IQR) (years)	34.9 (33.1- 38.4)
Gender	
• *Male*	3 (42.9)
• *Female*	4 (57.1)
Ethnicity	
• *White/Caucasian*	5 (71.4)
• *Black/Black British*	1 (14.3)
• *Asian/Asian British*	1 (14.3)
• *Other*	0 (0)

Table 185. Spirometry measures at V1 compared to V2 in the LOCA group.

Spirometry measures	LOCA V1 (n= 7)	LOCA V2 (n= 7)	P value
	N (%) unless stated otherwise		
FEV_1% (Mean ± SD) (n= 7)	91.3 ± 6.3	88.1 ± 12.8	0.38
FVC% (Mean ± SD) (n= 7)	95.8 ± 2.9	98.5 ± 7.6	0.35
FEV_1/FVC Median (IQR) (n= 7)	78.0 (72.9- 82.3)	68.9 (67.7- 77.5)	**0.02**
PEFR% (Mean ± SD) (n= 7)	99.2 ± 9.8	96.9 ± 23.9	0.75
FEV_1 <80%	0 (0)	1 (14.3)	NA
FVC <80%	0 (0)	0 (0)	
FEV_1 >80% and FVC <80%	0 (0)	0 (0)	
FEV_1/FVC <70%	0 (0)	4 (57.1)	NA
PEFR <75%†	0 (0)	1 (14.3)	NA

†Compared to best PEFR value or predicted PEFR if best is not available.

Figure 44. Lung function ratio at V1 compared to V2 in the LOCA group.

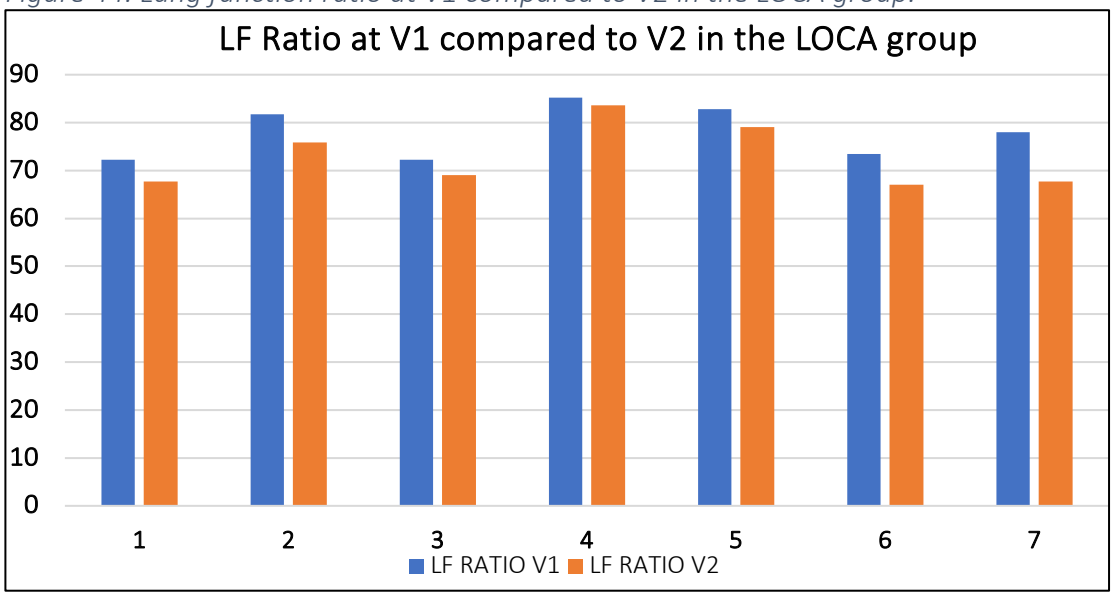


Table 186. Forced oscillation technique measures at V1 compared to V2 in the LOCA group.

FOT measures	LOCA V1 (n= 7)	LOCA V2 (n= 7)	P value
	N (%) unless stated otherwise		
R5 (Mean ± SD) (n= 7)	3.3 ± 0.8	4.0 ± 1.2	0.07
R5 <5	0 (0)	1 (14.3)	NA

Figure 45. Forced oscillation technique (R5) at V1 compared to V2 in the LOCA group.

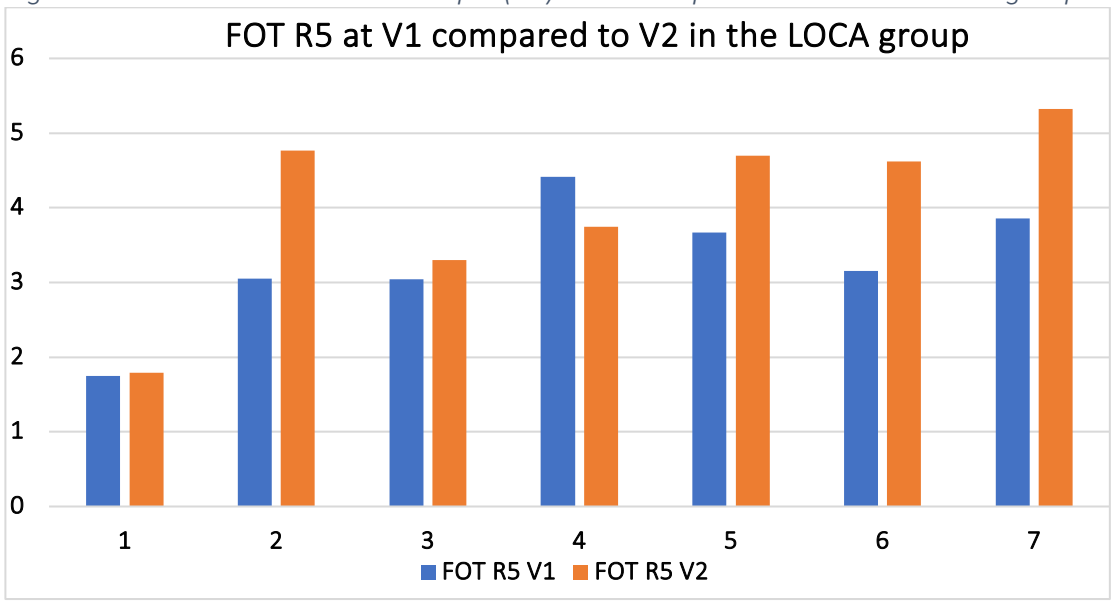


Table 187. Small airway measures at V1 compared to V2 in the LOCA group.

Small airway disease	LOCA V1 (n= 7)	LOCA V2 (n= 7)	P value
	N (%) unless stated otherwise		
$FEF_{25-75\%}$ (Mean ± SD) (n= 7)	82.2 ± 18.1	65.9 ± 23.9	0.02
R5-R20 (Mean ± SD) (n= 7)	0.5 ± 0.5	0.9 ± 0.5	0.18
$FEF_{25-75\%}$ <65%	1 (14.3)	3 (42.9)	NA
R5-R20 >1	2 (28.6)	3 (42.9)	NA
Small airway disease*	2 (28.6)	4 (57.1)	NA

**Defined as per Lipworth's criteria: Preserved FEV_1 (>80%) and (FEF_{25-75} <60% OR R5-20 >1 cmH2O/L/s).*

Table 188. Inflammatory biomarkers at V1 compared to V2 in the LOCA group.

Inflammatory measures	LOCA V1 (n= 7)	LOCA V2 (n= 7)	P value
	N (%) unless stated otherwise		
BEC Median (IQR) (n= 7)	0.36 (0.25- 0.49)	0.46 (0.17- 0.58)	0.71
FeNO Median (IQR) (n= 7)	54.0 (39.5- 92.0)	39.0 (30.0- 66.0)	0.10
SEC% Median (IQR) (n= 1)	20.5 (NA)	5 (NA)	NA
SNC% Median (IQR) (n= 1)	70.8 (NA)	61 (NA)	NA
SNC ≥65%	1 of 1 (100)	0 of 1 (0)	NA
Raised $T2^{APEX3}$	7 of 7 (100)	6 of 7 (85.7)	NA
• BEC ≥0.4 $x10^9$/L	3 of 7 (42.9)	4 of 7 (57.1)	NA
• FeNO ≥25 ppb	7 of 7 (100)	6 of 7 (85.7)	NA
• SEC ≥3%	1 of 1 (100)	1 of 1 (100)	NA
Raised $T2^{GINA}$	7 of 7 (100)	6 of 7 (85.7)	NA
• BEC ≥0.15 $x10^9$/L	6 of 7 (85.7)	5 of 7 (71.4)	NA
• FeNO ≥20 ppb	7 of 7 (100)	6 of 7 (85.7)	NA
• SEC ≥2%	1 of 1 (100)	1 of 1 (100)	NA

Table 189. Measures of infection at V1 compared to V2 in the LOCA group.

Infection status	LOCA V1 (n= 7)	LOCA V2 (n= 7)	P value
	N (%)		
CRP (> 10 mg/L) (n= 7)	1 (14.3)	2 (28.6)	NA
BNC (> 8 $x10^9$/L) (n= 7)	3 (42.8)	1 (14.3)	NA

Table 190. Acute Asthma Quality of Life Questionnaire at V1 compared to V2 in the LOCA group.

AAQLQ	LOCA V1 (n= 7)	LOCA V2 (n= 7)	P value
	N (%) unless stated otherwise		
AAQLQ overall score Median (IQR)	3.6 (3.0- 5.5)	5.9 (3.4- 6.6)	0.15
Minimum important difference			
• *No difference between visits (<0.5)*	3 of 7 (42.9)		
• *Minimum difference (≥0.5)*	2 of 7 (28.6)		
• *Moderate difference (>1.0)*	0 of 7 (0)		
• *Large difference (>1.51)*	2 of 7 (28.6)		
Symptoms score Median (IQR)	4.0 (3.8- 5.4)	6.2 (3.5- 6.8)	0.29
Minimum important difference			
• *No different between visits (<0.5)*	3 of 7 (42.9)		
• *Minimum difference (≥0.5)*	1 of 7 (14.3)		
• *Moderate difference (>1.0)*	1 of 7 (14.3)		
• *Large difference (>1.51)*	2 of 7 (28.6)		
Emotional score Median (IQR)	3.2 (2.4- 5.2)	5.0 (3.2- 6.6)	0.11
Minimum important difference			
• *No different between visits (<0.5)*	4 of 7 (57.1)		
• *Minimum difference (≥0.5)*	0 of 7 (0)		
• *Moderate difference (>1.0)*	2 of 7 (28.6)		
• *Large difference (>1.51)*	1 of 7 (14.3)		

Table 191. Acute Asthma Quality of Life Questionnaire at V3 compared to V2 and V1 in the LOCA.

AAQLQ	LOCA V1 (n= 5)	LOCA V3 (n= 5)	P value	LOCA V2 (n= 5)	LOCA V3 (n= 5)	P value
	Median (IQR)			Median (IQR)		
Overall score†	3.6 (3.5- 5.7)	6.5 (5.4- 6.5)	0.18	6.5 (5.9- 6.7)	6.5 (5.4- 6.5)	0.85
Symptoms score†	4.0 (3.8- 5.7)	6.2 (6.2- 6.3)	0.19	6.8 (6.2- 6.8)	6.2 (6.2- 6.3)	1
Emotional score†	3.2 (3.2- 5.2)	6.8 (4.4- 7.0)	0.18	6.6 (4.6- 6.6)	6.8 (4.4- 7.0)	0.86

†In those who completed V3.