DOCTOR OF PHILOSOPHY

Precision Medicine in Severe Asthma

Chan, Rory Kai-Yun

Award date:
2023

PRECISION MEDICINE IN SEVERE ASTHMA

RORY KAI-YUN CHAN

DEGREE OF DOCTOR OF PHILOSOPHY

MARCH 2023

UNIVERSITY OF DUNDEE

For my wife Caitlin and baby Rory

Contents

List of tables 7
List of figures 10
Acknowledgements 12
Declaration 14
Summary of thesis 17
Abbreviations 19
Chapter 1: Introduction & Literature Review 23
Precision medicine 24
Definition and epidemiology of severe asthma 26
Type 2 inflammation in asthma 27
Treatment recommendations for severe asthma 29
Pragmatic clinical perspective on biologics for severe refractory type 2 asthma 31
ABSTRACT 31
INTRODUCTION 31
EOSINOPHILIC ENDOTYPES 35
FENO-HIGH ENDOTYPES 39
ALLERGIC ENDOTYPES 41
TREATING T2 COMORBIDITIES 42
FURTHER CLINICAL CONSIDERATIONS 43
CONCLUSIONS 45
Targeting downstream type 2 cytokines or upstream epithelial alarmins for severe asthma 47
ABSTRACT 47
INTRODUCTION 47
DOWNSTREAM TARGETING OF IL-4/5/13 PATHWAYS 49
TEZEPELUMAB (ANTI-TSLP) 50
ASTEGOLIMAB (ANTI-ST2) AND ITEPEKIMAB (ANTI-IL33) 55
BRODALUMAB (ANTI-IL25) 56
PROPOSED BIOLOGIC AND ANTI-ALARMIN FLOWCHART 57
FURTHER CLINICAL CONSIDERATIONS 60
Chapter 2: Methods 64
Asthmatic participant selection 65
Airway measurements 66
Peak expiratory flow 66
Spirometry 66
Oscillometry 66

Mannitol challenge tests 67
Quality of life, asthma control and nasal symptom measures 67
Diary cards 67
Asthma quality of life questionnaire 68
Asthma Control Questionnaire 68
Total nasal symptom score 68
Hyposmia and global visual analogue scales 69
Peak nasal inspiratory flow 69
Fractional exhaled nitric oxide 69
Blood biomarkers 69
Peripheral blood measurements 69
Serum eosinophil derived neurotoxin 70
Imaging 70
Mucus plugging 70
Bronchial wall thickness 70
Quality control 71
Statistical methods 71
Illustrations 71
Chapter 3: Small Airways Dysfunction and Biologics in Severe Asthma 72
Impact of biologic therapy on the small airways asthma phenotype 73
ABSTRACT 73
INTRODUCTION 73
OMALIZUMAB 75
MEPOLIZUMAB, RESLIZUMAB AND BENRALIZUMAB 76
DUPILUMAB 78
TEZEPELUMAB 78
ITEPEKIMAB 78
CONCLUSIONS 81
Oscillometry bronchodilator response in adult moderate to severe eosinophilic asthma patients 82
Addendum 86
Repeatability of impulse oscillometry in patients with severe asthma 88
Interactions between spirometry and oscillometry in patients with moderate to severe asthma 94
Chapter 4: The impact of nasal polyps on clinical phenotype in severe asthma 101
Introduction 102

The impact of nasal polyps on clinical phenotype in moderate to severe asthma patients 104
ABSTRACT 104
INTRODUCTION 105
METHODS 106
RESULTS 107
DISCUSSION 111
Chapter 5: Correlating radiological and clinical features in moderate to severe asthma 114
Introduction 115
Clinical associations of mucus plugging in moderate to severe asthma 116
ABSTRACT 116
INTRODUCTION 117
METHODS 117
RESULTS 119
DISCUSSION 125
Impaired airway oscillometry is associated with bronchial wall thickening in persistent asthma 129
ABSTRACT 129
INTRODUCTION 129
METHODS 130
RESULTS 131
DISCUSSION 133
Chapter 6: Effect of benralizumab on airway hyperresponsiveness in severe asthma 135
Efficacy of biologic therapy on airway hyperresponsiveness in persistent asthma 136
INTRODUCTION 136
OMALIZUMAB (ANTI-IGE) 137
MEPOLIZUMAB (ANTI-IL5) 137
TRALOKINUMAB (ANTI-IL13) 138
TEZEPELUMAB (ANTI-TSLP) 139
SUMMARY AND OTHER CLINICAL CONSIDERATIONS 139
Eosinophil depletion with benralizumab is associated with attenuated mannitol airway hyperresponsiveness in severe uncontrolled eosinophilic asthma 142
ABSTRACT 142
INTRODUCTION 143
METHODS 143
RESULTS 148
DISCUSSION 157

Conclusion of thesis.....161
Publications arising from this thesis.....162
Presentations arising from this thesis.....164
References.....165

List of tables

Table 1. Effects of biologics on key patient outcomes and type 2 inflammatory biomarkers

Table 2. RCT data on effects of anti-alarmin therapy and downstream cytokine blockade on key outcomes in patients with severe asthma

Table 3. Mean Improvements in AER, FEV_1, FeNO, and asthma control with tezepelumab or dupilumab versus placebo from phase 3 trials

Table 4. Summary of current evidence base for the effect of biologics on forced expiratory flow rate between 25 and 75% of forced vital capacity (FEF_{25-75})

Table 5. Baseline patient demographics

Table 6. Mean absolute and percentage differences and standardised response means for pre- and post-bronchodilator oscillometry and spirometry measurements

Table 7. Comparisons of spirometry, oscillometry, asthma control and type 2 biomarkers according to presence or absence of bronchodilator response using FEV_1 or oscillometry criteria

Table 8. Baseline patient demographics

Table 9. Mean change, coefficient of variation and biological variability in pulmonary function, ACQ and type 2 biomarkers over time

Table 10. Overall patient demographics

Table 11. Significant differences in spirometry, oscillometry, type 2 biomarkers, asthma control and OCS requiring exacerbations comparing FEF_{25-75} ≥60%, R5-R20 <0.10kPa/L/s versus FEF_{25-75} ≥60%, R5-R20 ≥0.10kPa/L/s; and FEF_{25-75} <60%, R5-R20 <0.10kPa/L/s versus FEF_{25-75} <60%, R5-R20 ≥0.10kPa/L/s

Table 12. Significant differences in spirometry, oscillometry, type 2 biomarkers, asthma control and OCS requiring exacerbations comparing FEF_{25-75} ≥60%, AX <1.0kPa/L versus FEF_{25-75} ≥60%, AX ≥1.0kPa/L and FEF_{25-75} <60%, AX <1.0kPa/L versus FEF_{25-75} <60%, AX ≥1.0kPa/L

Table 13. Phase 3 trials comparing type 2 biologics versus placebo where endoscopic nasal polyp score (NPS 0-8) was the co-primary end point and 22 item sino-nasal outcome test (SNOT-22 0-110) was a secondary outcome.

Table 14. Demographic Data and Comparisons in Type 2 Biomarkers, Lung Function and Asthma Control

Table 15. Comparisons between groups according to use of high dose ICS and absence of allergic rhinitis in group A

Table 16. Overall patient demographics

Table 17. Comparisons in pulmonary function, type 2 biomarkers, asthma control and severe exacerbations between asthma patients with or without mucus plugging

Table 18. Comparisons in pulmonary function, type 2 biomarkers, asthma control and severe exacerbations excluding bronchiectasis patients

Table 19. Multiple linear regression analyses using spirometry, exacerbations and type 2 biomarkers as dependent variables and mucus plug score as the independent variable

Table 20. Odds ratios (95%CI) for spirometry, type 2 biomarkers and exacerbations in their association with the presence of mucus plugging score ≥1 using logistic regression modelling adjusted for confounding variables

Table 21. Overall patient demographics

Table 22. Adjusted odds ratios (95%CI) for spirometry, oscillometry, asthma control, nasal polyposis, exacerbations, and type 2 biomarkers associating with wall area thickness ≥50%

Table 23. Pearson correlations for WA% measurements in relation to individual bronchopulmonary segments

Table 24. Baseline patient demographics

Table 25. Mean differences, coefficients of variation and biological variability values between pre and post run-in (baseline).

Table 26. Mean values for mannitol PD_{10}, RDR, spirometry, oscillometry, ACQ, mini-AQLQ and type 2 biomarkers at weeks 0, 2, 4, 8, 12 and 24.

Table 27. Mean individual components of the mini-AQLQ response to benralizumab

Table 28. Comparison of baseline demographics, asthma control, type 2 biomarkers and FEV1 in patients whose mannitol PD_{10} response exceeded 1.0 doubling doses after 3 doses of benralizumab versus those who did not.

Table 29. Mean peak expiratory flow rate, patient subjective symptoms and reliever use in 7 days prior to respective visit

List of figures

Figure 1. Relationship between type 2 inflammation with asthma and relevant comorbidities

Figure 2. Proposed pragmatic clinical decision-making algorithm for the management of uncontrolled severe refractory type 2 asthma in relation to current available biologics

Figure 3. Commonly occurring patterns of type 2 inflammation in relation to choosing optimal biological therapy for severe uncontrolled asthma

Figure 4. Effects of biologic and anti-alarmin therapies on upstream epithelial alarmins, downstream cytokines and type 2 biomarkers in the context of key patient clinical outcome measures in severe asthma

Figure 5. Type 2 biomarker pivot in regard to FeNO and blood eosinophils depicting the proposed clinical flowchart in severe refractory asthma despite optimal inhaled and oral therapies

Figure 6. Comparisons in type 2 biomarkers and oscillometry in asthma patients with or without NP

Figure 7. Study flowchart

Figure 8. Distribution of mucus plug scores in the overall moderate to severe asthma patient cohort

Figure 9. Percentage prevalence of mucus plugging according to pulmonary lobe depicted by heatmap

Figure 10. Adjusted odds ratios (95%CI) for clinical outcomes associated with the presence of mucus plugging

Figure 11. Adjusted odds ratios (95%CI) for associations between bronchial wall thickness with FEV_1, oscillometry, severe exacerbations and nasal polyposis

Figure 12. Mean doubling dose difference in airway challenge agent for various biologics in asthma.

Figure 13 Tezepelumab and benralizumab have demonstrated effectiveness in attenuating airway hyperresponsiveness in patients with persistent asthma through different pathways.

Figure 14 Patient disposition

Figure 15 Study flowchart

Figure 16 Individual doubling differences for PD10 and RDR after 12 weeks of benralizumab therapy as change from baseline.

Figure 17 Mean doubling differences (95%CI) for PD10 and RDR at serial timepoints after benralizumab therapy.

Figure 18 Mean improvements (95%CI) in ACQ and mini-AQLQ scores at various timepoints after benralizumab therapy.

Figure 19 Suppression of peripheral blood eosinophils and eosinophil derived neurotoxin post benralizumab

Figure 20 Absolute change in peak expiratory flow compared to baseline as means and 95%CI

Acknowledgements

I started my PhD studies in August 2019 immediately prior to the first wave of the COVID-19 pandemic. At the peak of the pandemic, morale in the UK was low and there was significant uncertainty around whether I could gain traction with my asthma research since my main clinical trial (final chapter) could not commence in light of the serious clinical situation. My nursing and medical colleagues were struggling with fatigue on the National Health Service frontline, and I subsequently volunteered to return to clinical practice in April 2020 for 5 months.

When I returned to research in September 2020 my academic situation did not significantly improve. I attempted numerous times to begin my clinical trial but was impeded by second- and third pandemic waves, associated with government mandated national lockdowns. Just prior to surrendering and returning to NHS clinical practice, there were first signs that the pandemic was fizzling out and my clinical trial officially started in April 2021, 20 months after my PhD start date.

To ensure timely completion of my clinical trial and this thesis submission, there were months consisting of 15 patient trial visits per week, each lasting 4 hours, with the constant worry of a further lockdown looming above me like a dark cloud. This was in addition to volunteer recruitment and background data collection, analysis, illustration and figure creation, manuscript drafting and paperwork for the other studies presented in this thesis.

Looking back, I would never have been able to formulate this body of work without the generous help of others. In particular I would like to thank:

Professor Brian Lipworth, my postgraduate supervisor, for his mentorship, guidance and introduction to research. Without his recommendation, intellect, support, unwavering enthusiasm, and encouragement during my lowest point this project would never have been possible.

Dr Chris RuiWen Kuo, my predecessor as clinical research fellow, for his support upon becoming a clinical research fellow.

Ms Cally Smith and Mrs Deirdre Raeside, clinical research assistants, for their help in completing study visits for the BISA trial.

Dr Chary Duraikannu and Dr Mohamed Thouseef, consultant radiologists who helped provide radiology expertise for two studies within this thesis.

Ms Lesley McFarlane, senior technician and Ms Gwen Kennedy, core facilities manager for their help in performing eosinophil derived neurotoxin assays for the BISA trial.

All the volunteers who kindly participated in the BISA study.

Professor David Jackson and Dr David Connell, my external and internal viva examiners, for detailed reading of my thesis in totality and preparing for a very memorable and interesting discussion on my research over the last three years.

Professor Jacob George, my viva convenor, for meticulously organising my viva exam and related proceedings.

Professor James Chalmers, Professor Amelia Shoemark and Dr Ify Mordi, members of my thesis monitor committee, for helping me shape this thesis and guiding me over the years.

I would also like to thank my Dad and Mum for giving me opportunities they never had, and also Caitlin and our son Rory for their ongoing love, support and understanding. I will spend more time with both of you I promise.

Declaration

I declare that I am the author of this thesis, with all references consulted by me, and I have generated all the findings in this thesis. The work described here has not been previously submitted or accepted for another higher degree. My contribution to the totality of work described here is as follows:

All of the work described in this thesis was carried out during my time as a clinical research fellow with the Scottish Centre for Respiratory Research, University of Dundee under the supervision and tutelage of Professor Brian Lipworth.

I joined the department as a clinical research fellow in August 2019, initially contributing to my predecessor Dr Chris RuiWen Kuo's study as per the ethos of the department. Due to the COVID-19 pandemic I volunteered to return to the National Health Service back into my original role as a respiratory and general medical registrar between April and August 2020 inclusive (5 months). I resumed my research work in September 2020 but due to subsequent Scottish Government imposed lockdowns related to the pandemic, the BISA trial did not officially begin recruitment until April 2021.

During the pandemic lockdown months, I took the opportunity to use my time as efficiently as possible to perform literature reviews in addition to health informatics studies until government mandates allowed commencement of the benralizumab in severe asthma (BISA) clinical trial.

As part of the introduction of this thesis, I performed two literature reviews on biologic and anti-alarmin therapies in relation to precision medicine in severe asthma and prepared all versions of the manuscripts that led to peer reviewed publications.[1,2]

I subsequently carried out a retrospective analysis investigating the impact of nasal polyps on patient phenotypes in moderate to severe asthma, the idea of which was conceived by Prof Lipworth and myself. I collected all data, performed all analyses and prepared all versions of the manuscript leading to peer reviewed publication.[3]

As part of the introduction for the chapter 3 of this thesis, I performed a literature review looking at the effect of biologic therapies on the small airways in moderate to severe asthma.

I conceived the idea and together with Prof Lipworth, I drafted all versions of the manuscript leading to peer reviewed publication.[4]

My second study, assessing the repeatability of impulse oscillometry in severe asthma, was conceived by Prof Lipworth and myself. My role was in collecting and analysing all data prior to creating all versions of the manuscript leading to peer reviewed publication.[5]

The third study was a further retrospective study conceived by Prof Lipworth and myself examining the effects of combining oscillometry and spirometry in the assessment of moderate to severe asthma. My role was in organising, collecting and analysing all data before drafting all versions of the manuscript leading to peer reviewed publication.[6]

My fourth study was a prospective cohort study conceived by Prof Lipworth and myself comparing spirometry and oscillometry bronchodilator responses in severe eosinophilic asthma. This study included all patients who were enrolled in the BISA trial as well as screen failures. I carried out all the study visits, collected and analysed all data prior to producing all versions of the manuscript leading to peer reviewed publication.[7]

The fifth study was a retrospective analysis evaluating the effect of mucus plugging on patient phenotypic features in moderate to severe asthma. Following idea conception by Prof Lipworth and myself, I approached consultant radiologist Dr Chary Duraikannu who identified and quantified mucus plugging burden on pre-existing CT imaging in our asthma cohort. I was involved in collecting and analysing all data and drafting all versions of the manuscript leading to peer reviewed publication.[8]

The sixth study was a retrospective cohort study determining clinical associations with bronchial wall thickening in moderate to severe asthma. Dr Duraikannu and Dr Mohamed Thouseef identified and quantified bronchial wall thickness on pre-existing CT imaging. I conceived the idea and collected and analysed all data prior to writing all drafts of the manuscript leading to peer reviewed publication.[9]

My seventh and final study in this thesis was the phase IV clinical trial BISA which was conceived by Prof Lipworth, Dr Kuo and myself. Here, I was responsible for identifying and recruiting all the volunteers. Together with Ms Cally Smith, I personally performed all of the

clinical trial visits. I was involved in organising, collecting and analysing all data and writing all versions of the manuscript for peer reviewed publication.[10]

Rory Kai-Yun Chan

Summary of thesis

Although comprising a small portion of all asthmatics, patients with severe asthma utilise a disproportionate amount of healthcare resources, have significantly higher mortality and hospitalisation and reduced quality of life compared to those with milder disease.[11] Advances in recent years have witnessed the introduction of biologic agents that have improved patient outcomes with regards to exacerbation reduction, asthma control, quality of life and lung function.[12] At present, type 2 biomarkers, patient comorbidities and clinician and patient preference determine choice of biologic in those who are uncontrolled on optimal inhaled and oral therapies.

The aim of this PhD is to further our knowledge regarding specific aspects of precision medicine in severe asthma including less well studied areas such as small airways dysfunction, mucus plugging, bronchial wall thickness and airway hyperresponsiveness in the context of decisions surrounding biologic therapy.

The introduction of this thesis explores evidence from the currently available pivotal studies on biologic and anti-alarmin therapies in severe asthma. Chapter 3 summarises the current literature on the effect of biologics in small airways dysfunction (SAD) with the majority of the evidence base present from trials using spirometry defined SAD. This chapter also contains cohort studies demonstrating that oscillometry exhibits high repeatability over time and good sensitivity in detecting bronchodilator responses in severe asthma. Here, the effects of combining spirometry- with oscillometry-defined SAD are analysed to identify more severe asthma patients with worse disease control and more frequent severe exacerbations requiring oral corticosteroids.

Chapter 4 summarises the current evidence on biologics in chronic rhinosinusitis with nasal polyps (CRSwNP), a common comorbidity in patients with severe asthma. It also details a study with the aim of delineating phenotypic differences in asthma patients according to the presence or absence of CRSwNP.

In chapter 5, it is shown that mucus plugging, a common radiological feature of asthma, is associated with worse spirometry, greater type 2 inflammation, more frequent severe exacerbations, and higher Aspergillus fumigatus IgE titres. Furthermore, close associations

are demonstrated between bronchial wall thickening with severe exacerbations and lung compliance measured by oscillometry reactance area.

The final chapter of this thesis embodies the benralizumab in severe asthma (BISA) clinical trial (Eudract No. 2019-003763-22). In this study, it was shown for the first time that the anti-IL5Rα biologic benralizumab attenuates mannitol airway hyperresponsiveness, improves domiciliary peak flow and improves asthma control and quality of life in patients with severe eosinophilic asthma.

Abbreviations

A	asthma only
ABPA	allergic bronchopulmonary aspergillosis
ACQ	asthma control questionnaire
AERD	aspirin exacerbated respiratory disease
AHR	airway hyperresponsiveness
AOS	airwave oscillometry
AMP	adenosine monophosphate
AQLQ	asthma quality of life questionnaire
AR	allergic rhinitis
ATS	American Thoracic Society
AUC	area under curve
AwNP	asthma with nasal polyps
AX	area under reactance curve
BAL	bronchoalveolar lavage
BDP	beclomethasone diproprionate
BDR	bronchodilator response
BISA	benralizumab in severe asthma
BMI	body mass index
BV	biological variability
CI	confidence interval
CIU	chronic idiopathic urticaria
CROMO	sodium cromoglicate
CRSwNP	chronic rhinosinusitis and nasal polyposis
CT	computed tomography
CV	coefficient of variation
DD	doubling difference
EDN	eosinophil derived neurotoxin

EE	eosinophilic oesophagitis
EGPA	eosinophilic granulomatosis with polyangiitis
EOS	eosinophils
ERS	European Respiratory Society
FeNO	fractional exhaled nitric oxide
FEF_{25-75}	forced expiratory flow between 25 and 75% of forced vital capacity
FEV_1	forced expiratory volume in 1 second
Fres	resonant frequency
FVC	forced vital capacity
GINA	Global Initiative for Asthma
HRCT	high resolution computed tomography
ICS	inhaled corticosteroid
IgE	immunoglobulin type E
IL	interleukin
ILC2	type 2 innate lymphoid cells
IL4rα	interleukin 4 receptor alpha
IL5(rα)	interleukin 5 (receptor alpha)
INAH	intranasal antihistamine
INS	intranasal corticosteroid
IOS	impulse oscillometry
IQR	interquartile range
LABA	long-acting beta agonist
LAMA	long-acting muscarinic antagonist
LLLPB	left lower lobe posterior basal
LM	Lund Mackay
LTRA	leukotriene receptor antagonist
MCID	minimum clinical important difference
MDT	multidisciplinary team
Mini-AQLQ	mini-Asthma Quality of Life Questionnaire

MP	mucus plug
MPS	mucus plug score
MUC	mucin
NHS	National Health Service
NPS	nasal polyp score
NICE	National Institute for Health and Care Excellence
OAH	oral antihistamine
OCS	oral corticosteroid
OR	odds ratio
PBE	peripheral blood eosinophils
PC20	provocative concentration of histamine resulting in 20% drop in FEV_1
PD15	provocative dose of mannitol resulting in 15% drop in FEV_1
PD_{10}	provocative dose of mannitol resulting in 10% drop in FEV_1
pMDI	pressurised metered dose inhaler
ppb	parts per billion
R5	resistance at 5 Hz
R5-R20	difference between resistance at 5 and 20 Hz
R20	resistance at 20 Hz
RCT	randomised controlled trial
RDR	response dose ratio
RLLPB	right lower lobe posterior basal
ROC	receiver operator characteristics
SAD	small airways dysfunction
SD	standard deviation
SEA	severe eosinophilic asthma
SEM	standard error of means
SGRQ	St George's Respiratory Questionnaire
SPSS	Statistical Product and Service Solutions
SRM	standardised response means

T2	type 2 inflammation
Th2	T helper 2
THEO	theophylline
WA	wall area
X5	reactance at 5 Hz

Chapter 1: Introduction & Literature Review

Precision medicine

Over the past decade, the concept of precision medicine has gained traction in respiratory medicine. It encompasses accumulating a wide range of individualised data including clinical, lifestyle, genetic and biomarker information with the purpose of improving patient outcomes.[13]

A rewarding example of precision medicine can be found in the area of pulmonary oncology where the immunotherapy drug pembrolizumab, an inhibitor of programmed death 1 receptor, improves progression-free survival in patients with advanced non-small cell lung cancer with at least 50% expression of programmed death ligand 1 on immunohistochemical analysis.[14]

Precision medicine in severe asthma has led to the development of so-called “treatable traits” consisting of pulmonary, extra-pulmonary and behavioural risk factors that play important roles in the overall clinical outcome.[15] In a study of n=140 severe asthmatics where investigators assessed 26 individual treatable traits, there was a significant correlation between the number of treatable traits and health status measured by St George’s Respiratory Questionnaire (SGRQ).[15] Compared with usual care, focused targeting on treatable traits led to significantly greater improvements in Asthma Quality of Life Questionnaire and SGRQ. Furthermore, it is recognised that patients with raised blood or sputum eosinophils have more severe asthma, more frequent exacerbations and are more responsive to corticosteroids.[16] In this regard, the Unbiased Biomarkers in Prediction of Respiratory Disease Outcomes (U-BIOPRED) cohort defined clinical phenotypes using clustering analysis by identifying 8 clinico-physiological measurements including age of asthma onset, smoking pack years, body mass index, FEV_1 % predicted, FEV_1/FVC ratio, 5-point Asthma Control Questionnaire score, exacerbations in the past year and oral corticosteroid daily dose.[17] The resultant 4 reproducible clusters associated with different pathobiological pathways in asthma. However, it is recognised that precision medicine in severe asthma requires not only the identification of treatable traits but also includes causative mechanisms, the individual’s genome and their exposure to the environment.[16]

One of the aims of this thesis is to add to the literature base that small airways dysfunction is an important treatable trait in severe asthma. My other objectives include determining the relationship between mucus hypersecretion,[15] with severe asthma; and how the presence of bronchial wall thickness and nasal polyps affect the clinical phenotype of severe asthma patients.

Finally, despite airway hyperresponsiveness (AHR) representing a pivotal piece of the pathophysiological puzzle in persistent asthma, few studies on biologics have looked into its effect on AHR. The ultimate aim of this thesis is to evaluate the effect of benralizumab, an anti-IL5rα monoclonal antibody, on AHR in patients with severe asthma.

Prior to answering these important questions, a summary of the current evidence base regarding biologic and anti-alarmin therapies in severe asthma from landmark clinical trials is perhaps required.

Definition and epidemiology of severe asthma

Asthma is a heterogeneous disease that affects the respiratory system and is characterised by chronic airway inflammation and variable expiratory airflow limitation.[18] Although there is no single diagnostic test, asthma is usually investigated by a history of respiratory symptoms (wheeze, shortness of breath, chest tightness and cough that varies over time and in intensity), pulmonary function (peak expiratory flow, spirometry, forced oscillation technique, bronchodilator response and airway challenge testing) and the presence of type 2 inflammatory biomarkers (sputum and blood eosinophils; fractional exhaled nitric oxide and immunoglobulin E).[18]

The Global INitiative for Asthma (GINA)[19] describes asthma as uncontrolled if one or both of the following occurs: (1) suboptimal symptom control (frequent symptoms or reliever inhaler use; limited physical activity or night time wakening due to asthma) and (2) frequent exacerbations defined as ≥ 2 per year requiring oral corticosteroids (OCS), or serious exacerbations defined as ≥ 1 per year requiring hospitalisation. Difficult-to-treat asthma is defined as that which is uncontrolled despite the prescription of medium to high dose inhaled corticosteroids (ICS) with a second controller (which is usually a long acting beta agonist (LABA)) or with maintenance OCS, or that requires high dose therapy to maintain good symptom control and lower exacerbation risk.[19]

Severe asthma refers to a subgroup of difficult-to-treat asthma patients where asthma is uncontrolled despite adherence to maximal optimised high dose ICS-LABA and treatment of treatable traits, or that worsens when high dose therapy is reduced.[19] Difficult-to treat and severe asthma are thought to affect 17% and 3.7% respectively of adults patients over the age of 18 with asthma.[19]

Type 2 inflammation in asthma

Historically, the method for classifying asthma included an assessment of the patient's allergy status.[20] Allergic asthma is usually diagnosed on the basis of symptoms triggered by environmental exposures whilst allergy can be established by the presence of a positive skin prick test to common or specific allergens or by measuring serum levels of specific IgE.[18] Although IgE plays a pivotal role in the pathophysiology of allergic asthma, there is significant overlap in clinical presentation between allergic and non-allergic asthma in addition to the underlying inflammatory process which includes elevated levels of T helper type 2 (Th2) cells, mast cell activation and eosinophilic airway infiltration.[21-23]

Asthma can also be characterised by disease pathophysiology centred around the predominant pattern of cellular inflammation with presence of four subtypes: eosinophilic, neutrophilic, mixed eosinophilic and neutrophilic, and pauci-granulocytic (normal levels of eosinophils and neutrophils).[24] Elevated numbers of eosinophils in sputum, bronchoalveolar lavage or blood point towards eosinophilic asthma and is usually associated with a sputum eosinophil count >2.5% or peripheral blood eosinophil count ≥300 cells/μl irrespective of allergy status.[1] Neutrophilic asthma (sputum neutrophil count >65%) is uncommon and is thought to be less responsive to treatment with inhaled corticosteroids compared to eosinophilic asthma.[25]

The type 1 and type 2 helper (TH1 and TH2) cell immune response paradigm describes immune processes that are mediated by subpopulations of CD4+ T cells.[26] TH1 cells secrete interleukin-2 (IL2), interferon-γ (IFNγ) and lymphotoxin-α which primarily stimulate type 1 immunity largely driven by phagocytosis.[27] Type 2 immunity is characterised by elevated cytokine expression of IL4, IL5 and IL13 secreted by TH2 cells and are mediated by eosinophils, mast cells, basophils, type 2 innate lymphoid cells (ILC2), TH2 cells and IgE-producing B cells.[28,29] Type 2 immune responses are induced by parasitic helminths and are associated with allergic conditions such as asthma, food allergies and chronic rhinosinusitis with nasal polyps.[26,30]

In genetically predisposed individuals, the presence of allergens trigger the production of cytokines IL25, IL33 and thymic stromal lymphopoietin (TSLP) from the respiratory airway

epithelium.[31] These upstream alarmins induce inflammatory responses via downstream pathways that include type 2 (IL4, IL5 and IL13), TH1 and TH17 pathways.[32]

Treatment recommendations for severe asthma

Current guidelines suggest that patient education, inhaler technique and medication adherence are the foundations of good clinical practice when managing asthma patients.[19] Treatment of comorbidities including obesity, gastro-oesophageal reflux disease (GORD), chronic rhinosinusitis and obstructive sleep apnoea (OSA) should also be optimised.[33] Modifiable risk factors and triggers at home or work such as smoking, environmental exposures, allergens and medications (e.g. beta blockers and non-steroidal anti-inflammatory drugs) should be avoided if possible.[34]

Traditionally, adult asthma has been managed pharmacologically in a step-wise treatment algorithm beginning with as required short acting beta agonists (SABAs) and maintenance low dose inhaled corticosteroids (ICS).[35] If further asthma control is required this regimen is then escalated to maintenance medium and high dose ICS and/or the addition of a long acting beta agonist (LABA), leukotriene receptor antagonist (LTRA) or long acting muscarinic antagonist (LAMA).[35]

However, one possible concern with using as required SABA and fixed-dose ICS/LABA is that patients may become over-reliant on their SABA inhaler as this provides a more immediate sense of relief from their acute breathing impairment.[36] In the United Kingdom (UK), it has been suggested that an overuse of reliever therapy (SABA) and underuse of preventer inhaler (ICS) could be a contributor to increased mortality from asthma.[37]

In recent years, four key randomised controlled trials[38-41] (RCTs) have shown that anti-inflammatory reliever (AIR) therapy, by way of as required budesonide and formoterol (BUD/FM), was either noninferior or superior to budesonide on exacerbations. As opposed to maintenance and reliever therapy (MART) where, as the name suggests, an as required dose of BUD/FM is taken in addition to the maintenance BUD/FM dose, AIR refers to as required BUD/FM with no maintenance medication. Not only does this proposed solution allow patient controlled flexible dosing, it also ensures perfect concordance between reliever and controller use.[36]

If asthma control is not achieved with inhaled and oral therapies despite optimisation of the aforementioned factors, current guidelines suggest referral to a specialist respiratory centre for further investigation and management.[35]

Pragmatic clinical perspective on biologics for severe refractory type 2 asthma

ABSTRACT

Patients with severe refractory asthma present a challenging clinical conundrum for practising clinicians. Biologics that target key mediators in the type 2 (T2) inflammation cascade, including IL-4, IL-5, IL-13 and IgE, can be effective strategies for these patients. However, with various biologics available, choosing the optimal one for a particular patient becomes a nuanced decision. We propose a pragmatic algorithm which identifies the optimal biologic class for patients who have specific T2 disease endotypes. Patients with eosinophilic endotypes fare well with anti-IL5(rα) medications, comprising mepolizumab, benralizumab and reslizumab as they have been shown to reduce exacerbations in severe eosinophilic asthma by approximately 50%. In patients with FeNO-high endotypes, anti-IL4rα such as dupilumab is deemed to be most effective and has demonstrated a 47% reduction in asthma exacerbations although a recent indirect treatment comparison suggests further promising results. For patients with severe uncontrolled allergic asthma, anti-IgE (omalizumab) is effective and has been shown to confer a 25% reduction in asthma exacerbations. T2 comorbidities including chronic rhinosinusitis with nasal polyps, atopic dermatitis, chronic idiopathic urticaria and eosinophilic esophagitis are important to bear in mind prior to the prescription of biologics. Further head-to-head studies are indicated to compare biologics in patients with mixed endotypes according to peripheral blood eosinophils, FeNO and allergic status. The evidence strongly supports endotype-driven prescribing of biologics in order to achieve clinically relevant outcomes in severe refractory asthma and related comorbidities.

INTRODUCTION

Patients with severe uncontrolled asthma present a challenging clinical conundrum for practising clinicians due to their requirement for extensive diagnostic evaluation, high consumption of healthcare resources and heavy symptom burden.[42] Global Initiative for Asthma (GINA) defines severe asthma as uncontrolled despite adherence with maximal optimised therapy (step 4 or 5) and treatment of contributory factors, or that worsens when high dose treatment is decreased, affecting an estimated 3.7% of patients with asthma.

Type 2 (T2) inflammation asthma is primarily driven by various cytokines including IL-4, IL-5, and IL-13 and these in turn regulate the production of quantifiable biomarkers, namely IgE, eosinophils and fractional exhaled nitric oxide (FeNO) [figure 1]. It is thought that despite optimised inhaled corticosteroid (ICS) therapy many asthmatics have persistent airway T2 inflammation with this cohort of patients being older and having more severe disease.[43]

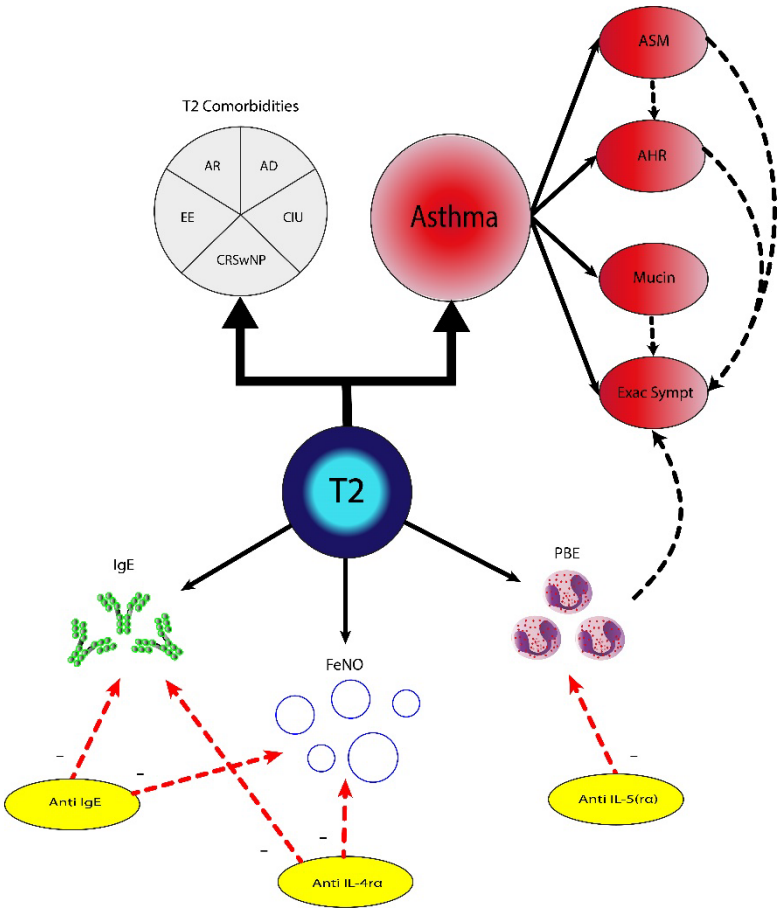

Figure 1 *Activation of T2 inflammation elevates levels of IgE, FeNO and PBE. These biomarkers are targeted by various biological therapies as depicted. Relationship between T2 inflammation with asthma and relevant comorbidities shown. AD – atopic dermatitis; AHR – airway hyperresponsiveness; AR – allergic rhinitis; ASM – airway smooth muscle; CIU – chronic idiopathic urticaria; CRSwNP – chronic rhinosinusitis with nasal polyps; EE – eosinophilic esophagitis; Exac – exacerbations; FeNO – fractional exhaled nitric oxide; IgE – immunoglobulin type E; IL – interleukin; PBE – peripheral blood eosinophils; Sympt – symptoms; T2 – type 2 inflammation.*

This article is not intended to be an exhaustive systematic review, nor will it explore non-T2 asthma and the follow-up decisions surrounding biological therapies such as stopping and switching decisions, as these have already been covered in detail elsewhere.[44-47] Instead its purpose is to provide a focussed pragmatic real-life practice guide for physicians based on current available guidance on biological therapies with particular reference to common T2

biomarkers. This is admittedly a challenging feat as most of the evidence is based on trials that were restricted to a specific endotype appropriate to the molecular target of the treatment and/or had inconsistent eligibility criteria that excluded certain populations of interest.[48]

It is always prudent to confirm the original asthma diagnosis.[49] Secondly, optimisation of inhaler technique, medication adherence, and management of comorbidities, modifiable risk factors and psychosocial circumstances is mandatory. For severe uncontrolled asthma, discussion at a severe asthma multidisciplinary team (MDT) should occur as there is growing evidence that this significantly reduces asthma-related hospital admissions and hospital days.[50] Indeed, our Tayside severe asthma MDT have meetings on a weekly basis.

In patients with T2 asthma, monoclonal antibodies targeting immunoglobulin type E (IgE), interleukin 4 receptor alpha (IL4rα) and interleukin 5 (IL5) are attractive therapeutic options as they reduce exacerbation rate and oral corticosteroid (OCS) dose requirement, as well as improve quality of life, pulmonary function and symptom control to varying degrees (Table 1).[51-53] This begs the question of which biologic is best suited to an asthmatic patient based on their particular disease endotype. Peripheral blood eosinophils (PBE), FeNO and allergic status are the most commonly utilised T2 biomarkers in clinical practice for assessing asthma and assisting in generating specialist decisions. Here we propose a simplified clinical algorithm to assist practising clinicians in determining the optimal biologic depending on the specific combination of T2 biomarkers in patients presenting with severe uncontrolled asthma based on common endotypes (figures 2 and 3).

Table 1 *Effects of biologics on key patient outcomes and type 2 inflammatory biomarkers*

MAb	Exac	FEV_1	ACQ/QoL	OCS sparing	PBE	IgE	FeNO
Anti-IL5	+++	+	+	++	++	-	-
Anti-IL5Rα	+++	+	+	++	+++	-	-
Anti-IL4rα	+++	++	+	++	-	++	++
Anti-IgE	++	+	+	N/A	+	+/-[#]	+

*ACQ = asthma control questionnaire; Exac = exacerbations; FeNO = fractional exhaled nitric oxide; FEV_1 = forced expiratory volume in 1 second; IgE = immunoglobulin type E; IL = interleukin; MAb = monoclonal antibody; PBE = peripheral blood eosinophils; QoL = quality of life; number of "+" symbols denotes degree of positive effect; *evidence for OCS sparing effect of Omalizumab is equivocal; # Omalizumab paradoxically elevates bound total and specific IgE levels but reduces free IgE.*

There is only one study where it is possible to estimate the relative prevalence of different T2 endotypes as enrolment was independent of biomarkers. Here the relative prevalence of endotypes was shown to be 42% for PBE ≥150/µl, FeNO ≥25ppb; 30% in PBE ≥150/µl, FeNO <25ppb; and 9% in PBE <150/µl FeNO ≥25ppb; while the remaining 19% had PBE <150/µl and FeNO <25ppb.[54] In essence, a large proportion (72%) of patients with severe asthma appear to have an eosinophilic endotype, albeit using a rather low cut point of ≥150/µl. This breakdown did not factor in the presence or absence of an allergic endotype. Furthermore, one recent retrospective observational cohort analysis demonstrated that 34% of severe asthma patients have an eosinophilic endotype using the more clinically relevant cut-point of 300/µl.[55] Recent data from a large global real-life study demonstrated that, based on a combination of clinical and biomarker variables, 84% of severe asthma patients most likely have an eosinophilic endotype with a further 8% having likely eosinophilia and <2% having a non-eosinophilic endotype.[56] Pointedly, this study included the impact of oral corticosteroids and temporal variability of blood eosinophil counts as potential confounding variables. Another recent study validated these results by showing that 83% of difficult-to-manage asthma patients have had a blood eosinophil count ≥300 cells/µl over the past decade.[57]

Allergic asthma (defined as at least one positive allergen-specific test) is widely regarded as the most common endotype with a prevalence of around 56%.[58] The Severe Asthma Research Program (SARP) study estimated that the proportion of severe asthma patients with a

negative skin prick test varied between 17 and 34%,[59] in keeping with the U-BIOPRED cohort's approximation.[17]

For the purposes of this review article, allergy in keeping with the Omazilumab label indication is defined as a total serum IgE ≥30 IU/mL and ≥1 perennial aeroallergen specific IgE ≥0.35 kU/L at baseline.[60] However in real life clinical practice, our Tayside severe asthma multidisciplinary team (MDT) meeting would only designate a patient with a total serum IgE ≥100 IU/mL and ≥2 aeroallergen specific IgE ≥0.35 kU/L or positive skin prick tests at baseline to be a clinically relevant allergic endotype.[61] This definition is based on our regional experience that has been pragmatically adapted from clinical practice but we duly appreciate that most of the studies and evidence base use the former criteria for defining allergy. Similarly, we would only classify patients into an eosinophilic endotype if their PBE count exceeded 300/µl, ideally over 2 different time points in the preceding 6 months. Clinicians should recognise that significant variability of blood eosinophils in patients with severe asthma exists, further stressing the importance of repeat measurements over time for the appropriate allocation of therapeutic interventions.[62] At this juncture it is also important to point out that the presence of raised FeNO is highly dependent on adherence to ICS therapy or the use of oral corticosteroids (OCS), both of which suppress FeNO. For the purpose of this review, we will adopt a pragmatic cut off of ≥25ppb while taking ICS to denote a patient with a high FeNO endotype.

EOSINOPHILIC ENDOTYPES

A recent Cochrane review indicates that the three anti-IL5(rα) agents – mepolizumab, benralizumab and reslizumab – reduce rates of clinically significant asthma exacerbations by approximately 50% in patients with severe eosinophilic asthma on standard of care.[63] Furthermore, they were shown to produce a small (80 – 110ml) but statistically significant improvement in forced expiratory volume in 1 second (FEV_1), although it is perhaps worth noting that the minimum clinical important difference (MCID) is traditionally considered to be 230ml.[64] Patients also experienced modest improvements in their asthma control questionnaire (ACQ) and asthma quality of life questionnaire (AQLQ) but these were both also below the conventional MCID of 0.5.[65] In the UK, the National Institute for Health and Care Excellence (NICE) guidance for mepolizumab and benralizumab suggest at least 4 severe exacerbations needing systemic steroids along with PBE ≥300 cells/µl in the past year or

continuous OCS requirement over the previous 6 months. Reslizumab and benralizumab are also indicated in UK for patients with PBE ≥400/µl and at least 3 exacerbations in the past 12 months.

The more common endotypes discussed in this article are depicted in figure 3: PBE-high, FeNO-high, allergic (endotype 1); PBE-high, FeNO-high, non-allergic (endotype 2); PBE-high, FeNO-low, non-allergic (endotype 3); and PBE-low, FeNO-high and allergic (endotype 4). Patients with elevated PBE comprising endotypes 1-3 likely experience most benefit from anti-IL5(rα) therapy as eosinophilic proliferation, maturation and survival are governed by IL5.[66] Exploratory modelling of baseline characteristics of patients in phase 3 studies support substantial reductions in the rate of severe exacerbations with mepolizumab in patients with higher PBE counts.[53,67] Likewise, higher PBE counts predicted response in patients with severe eosinophilic asthma (SEA) treated with reslizumab or benralizumab.[68,69] Moreover, real world mepolizumab data suggests more impressive results compared to randomised controlled trials on reduction in exacerbations, hospitalisations along with an improvement in ACQ score of 2.0 points at six months which far exceeds MCID of 0.5, although the placebo effect should be considered when interpreting these data.[70]

Therefore, for any of the eosinophilic endotypes defined by PBE ≥300/µl, we would generally propose anti-IL5(rα) therapy as first line unless there was a specific reason otherwise (figure 2). This is based on the current evidence suggesting a higher exacerbation risk reduction with either anti-IL5(rα) (50%) or anti-IL4rα (47%) versus anti-IgE therapy (25%). Our tentative position here is that until there is good evidence showing reductions in airway eosinophilia from sputum or bronchial biopsy with anti-IL4rα, we would proffer a degree of caution in advocating dupilumab as equal first line therapy with anti-IL5(rα) for such patients despite similar reductions in exacerbations. The following discussion delves deeper into the individual eosinophilic endotypes and implications for biologic therapy.

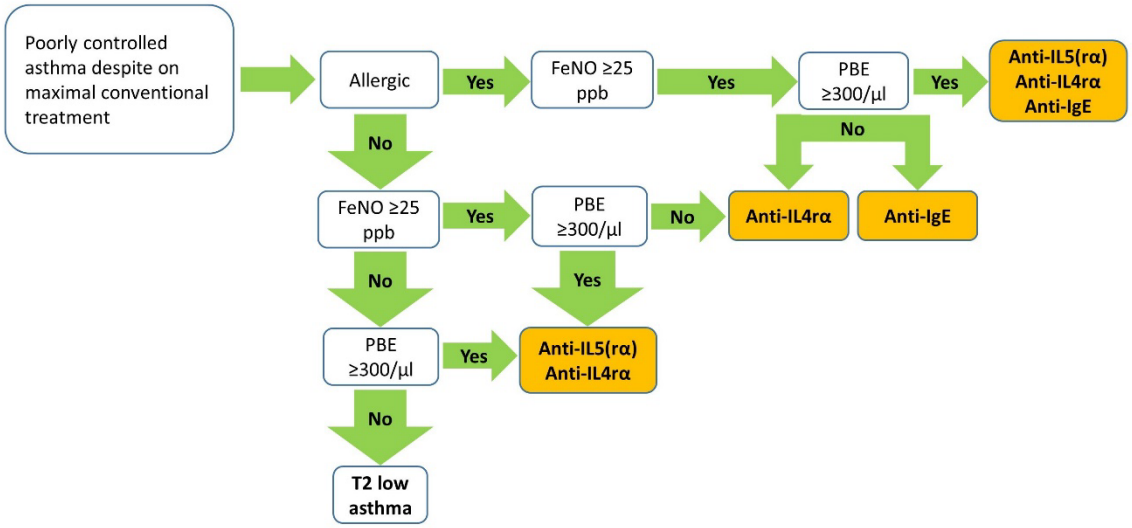

Figure 2 *Proposed pragmatic clinical decision-making algorithm for the management of uncontrolled severe refractory T2 asthma in relation to the current available biologics. FeNO – fractional exhaled nitric oxide; IL – interleukin; µl – microlitre; PBE – peripheral blood eosinophils; ppb – parts per billion.*

For endotype 1, any of the monoclonal antibodies directed against IL5(rα), IL4rα or IgE might in theory be considered equivalent first line options. However, currently available evidence seems to suggest a greater decrease in asthma exacerbation rates and OCS dose requirement in patients treated with anti-IL5(rα) or anti-IL4rα compared to those on anti-IgE.[52,63,71] Therefore, in the absence of any defining comorbidities, our MDT would recommend anti-IL5(rα) or anti-IL4rα as first line, with anti-IgE as second line in patients with endotype 1 (figure 2). In real life clinical practice, the choice of biologic in patients with this endotype would rest upon physician experience and preference, informed patient choice, cost and presence of any other relevant comorbidities, which are explored in more detail later. For example, patients leading a busy life might prefer the convenience of taking maintenance therapy with benralizumab every 8 weeks rather than dupilumab every 2 weeks.

Similarly, for endotype 2, evidence seems to support that either anti-IL5(rα) or anti-IL4rα could be considered first line therapy. For instance, pooled analysis of the benralizumab trials revealed that it maintains its effect on exacerbation reduction and lung function improvement for patients with SEA irrespective of allergic status.[72] It is worth noting that in this analysis, allergy was defined with a perhaps more clinically relevant serum total IgE cut-off of ≥150 kU/L.

To determine what actually constitutes clinically relevant eosinophilia, closer examination of a secondary analysis of the pivotal benralizumab trials reveals a so-called sweet spot for exacerbation rate reduction and FEV_1 improvement relative to placebo that appears to occur around PBE ≥300/µl[69] when plotted as a continuous variable. For instance, in the comparison between benralizumab 30mg q8wk and placebo, patients with PBE ≥300/µl and ≥3 exacerbations in the prior year experienced a relative exacerbation rate reduction of 55% and FEV_1 improvement of 252ml (above MCID of 230 ml).

In a post-hoc analysis of the pivotal dupilumab trials, using 200mg q2wk, exacerbations were reduced by 68% in patients with PBE ≥150/µl, FeNO ≥25ppb as opposed to 33% in patients with PBE ≥150/µl, FeNO <25ppb.[73] This infers that dupilumab could potentially be more effective in patients with endotypes 1 and 2 with high FeNO rather than those with endotype 3 with low FeNO. Unfortunately, no data were available for dupilumab stratified at PBE ≥300/µl according to FeNO ≥25ppb vs <25ppb which in our opinion would have been more informative. In this regard, a prototype asthma attack risk scale centred on PBE and FeNO has recently been designed,[74] demonstrating an association between increased exacerbation risk with thresholds of FeNO ≥25ppb and PBE≥300/µl. Prospective head to head trials would be required to assess whether anti-IL4rα or anti-IL5(rα) is more effective first line treatment for patients with both FeNO ≥25ppb and PBE ≥300/µl in endotypes 1 and 2. In the same post-hoc analysis for patients on mepolizumab with PBE ≥150/µl, exacerbation rate was reduced by 62% for FeNO ≥25ppb but only 36% for <25ppb.[73] Mepolizumab also resulted in modest FEV_1 improvements (122ml for ≥25 ppb and 101ml for <25ppb) in patients with PBE ≥150/µl, albeit this was below MCID.[64] For patients on mepolizumab with PBE ≥300/µl the exacerbation rate reduction was 62% for FeNO ≥25ppb and 53% for <25ppb, in keeping with the lack of effect of IL5 signalling on FeNO.

For endotype 3 i.e. PBE-high, FeNO-low and non-allergic, one might not expect patients to experience significant benefit from anti-IL4rα therapy as it acts on both IL4 and IL13, the latter of which regulates FeNO.[75] However, the aforementioned data[73] still implied a 33% reduction in exacerbation rate which might be clinically worthwhile. A key limitation here is the absence of available data for patients on dupilumab with PBE ≥300/µl according to FeNO ≥ or <25ppb. Nonetheless in the primary analysis[52] dupilumab 300mg q2wk produced a 67% exacerbation reduction in those with PBE ≥300/µl irrespective of FeNO, perhaps supporting a

recommendation that both anti-IL5(rα) or anti-IL4rα therapy may be considered as suitable first line options for endotypes 1, 2 and 3.

Despite the promising results seen with anti-IL5(rα) therapy, recent data suggests that 43% of patients who fulfil the current approved treatment criteria are so-called suboptimal responders.[76] Sputum analysis in this subset of patients suggests a possible underlying autoimmune mediated aetiology related to the presence of anti-eosinophil peroxidase IgG, with a caveat that further evaluation is required before this can be considered as part of routine practice.

FENO-HIGH ENDOTYPES

In addition to endotypes 1 and 2, the FeNO-high endotype also includes patients with the PBE-low, FeNO-high, allergic endotype 4. Patients with either of these three FeNO-high endotypes would in theory be expected to have a favourable response to anti-IL4rα therapy as FeNO is closely regulated by IL13,[75] however the results of the pivotal trials with tralokinumab and lebrikizumab which block IL13 signalling were equivocal.[77,78] In STRATOS 1 and 2 investigating tralokinumab, the overall baseline FeNO concentration for both the placebo and treatment arms was borderline at 30ppb.[77] This infers that a significant proportion of patients had FeNO levels less than the standard cut point of 25ppb and this may be a potential reason for the lack of response seen with tralokinumab therapy in the overall analysis. When analysing only patients with FeNO ≥37ppb, there was a significant AER reduction amounting to 44% (95%CI 6 – 66%) compared to placebo in STRATOS 1 but not in STRATOS 2. However, the large confidence interval suggests great heterogeneity in response between individual patients which in turn might depend on an unknown variable that was not measured. In STRATOS 1, it is perhaps worth mentioning that improvements in AER in the FeNO-high group were accompanied by improvements in pre-bronchodilator FEV_1, ACQ and AQLQ.

In LAVOLTA I and II, investigating lebrikizumab, the median baseline FeNO was 28ppb and 24ppb respectively in the treatment arms.[78] This borderline value might, at least in part, contribute towards an explanation as to why lebrikizumab did not meet its primary endpoint of AER reduction in the overall analysis. In LAVOLTA I, patients who were biomarker-high

defined as PBE ≥300 cells/µl or periostin ≥50ng/ml, experienced significant AER reductions versus placebo whereas this was equivocal in the biomarker-low group. In LAVOLTA II, there was a trend towards AER reduction with lebrikizumab in the biomarker-high analysis although this was not statistically significant (p=0.06). This difference could perhaps be explained by biomarker-high patients in LAVOLTA II having a lower baseline exacerbation frequency compared to those in LAVOLTA I, potentially translating into less room for improvement.

This in turn suggests that blocking signalling of both IL4 and IL13 with dupilumab might be required to improve asthma control.[79] In the post-hoc analysis of the pivotal dupilumab trials, exacerbations were reduced by 39% in patients with PBE <150/µl, FeNO ≥25ppb.[73] Although not statistically significant due to small sample size, this finding contrasted the absence of therapeutic effect seen with mepolizumab in this endotype where there was only a 6% reduction. Intriguingly, in an exploratory post-hoc analysis of dupilumab 300mg q2wk[52] for patients with PBE ≥150/µl, FeNO <25ppb there appeared to be discordance in terms of a significant reduction in exacerbations but no improvement in FEV_1 relative to placebo, whilst in patients with PBE <150/µl, FeNO ≥25ppb effects of dupilumab were concordant on both exacerbations and FEV_1. In another post-hoc analysis dupilumab showed equivalent efficacy in allergic and non-allergic asthma,[60] although the definition of allergy was tenuously based on total serum IgE ≥30 IU/mL and ≥1 perennial aeroallergen specific IgE ≥0.35 kU/L. Notably, no comparison of response was made across a range of IgE cut points. Nevertheless, anti-IL4rα would be a suitable option for patients with endotype 4 as we appreciate that most of the studies commonly define allergy using these criteria. Taken together this clearly emphasises the importance of measuring both PBE and FeNO in severe asthma before making an informed decision regarding tailored biologic therapy.

Interestingly, in a systematic review and meta-analysis of real-life clinical studies, mepolizumab (7 studies, n=363, moderate evidence) but not benralizumab (3 studies, n=179, low evidence) was associated with reductions in FeNO levels.[80] Significant heterogeneity was detected in individual effect sizes with benralizumab although this was found to be unrelated to baseline blood eosinophil counts. In this regard, a real-world study found that mepolizumab and benralizumab do not confer a differential response according to baseline FeNO level with this relationship preserved even in patients with the highest baseline FeNO (≥75ppb) levels.[81] This is in keeping with findings from the DREAM study investigating

mepolizumab which showed no additional improvements in annual exacerbation rates comparing baseline FeNO levels of <50ppb and ≥50ppb versus placebo.[53] However in the aforementioned post hoc analysis of DREAM, investigators detected a greater absolute annual exacerbation reduction of 62% for patients with PBE ≥150 cells/µl and FeNO ≥25ppb compared to 36% for those with PBE ≥150 cells/µl and FeNO <25ppb.[73] Although the 95% confidence intervals overlapped for this sub-analysis, this finding is worth exploring further in the future and for this reason, anti-IL5(rα) should be considered in patients who exhibit both blood eosinophilia and high FeNO.

Although there are no head to head trials comparing various biologics for the treatment of common T2 asthma endotypes, a recent indirect treatment comparison using 14 randomised controlled trials demonstrated that dupilumab was associated with a significantly greater reduction in annualised severe asthma exacerbation rate (26% greater reduction versus omalizumab and 28 – 54% versus anti-IL5(rα)).[82] A 60 – 140ml improvement in FEV_1 was also seen with dupilumab versus the other biologics although this is below the MCID of 230ml.

ALLERGIC ENDOTYPES

Anti-IgE is a viable alternative for patients with endotypes 1 and 4 as a 2014 Cochrane review evaluating 25 randomised trials using omalizumab demonstrated a 25% asthma exacerbation reduction as well as a significant ICS sparing effect.[71] Humbert et al showed in a retrospective real life analysis that omalizumab is an effective treatment option for severe allergic asthma irrespective of blood eosinophil count.[83] Furthermore, post hoc analysis of an omalizumab randomised controlled trial showed that lower baseline IgE concentrations were associated with a smaller benefit in exacerbation reduction and improvement in quality of life.[84] In another prospective placebo controlled trial omalizumab produced 39% greater relative exacerbation reduction in patients with FeNO ≥19.5ppb vs <19.5ppb and a 23% greater reduction comparing PBE ≥260/µl vs <260/µl.[85] Although anti-IgE therapy is a suitable treatment for patients with endotypes 1 and 4, it may be desirable to consider the other biologics first based on current evidence.

We wish to highlight that the PBE-low, FeNO-low, allergic endotype has deliberately been ommitted from figures 2 and 3 as in our clinical experience this is an uncommon clinical

pattern. We would advocate an interval repeat measurement of PBE in such cases to exclude a false negative result.

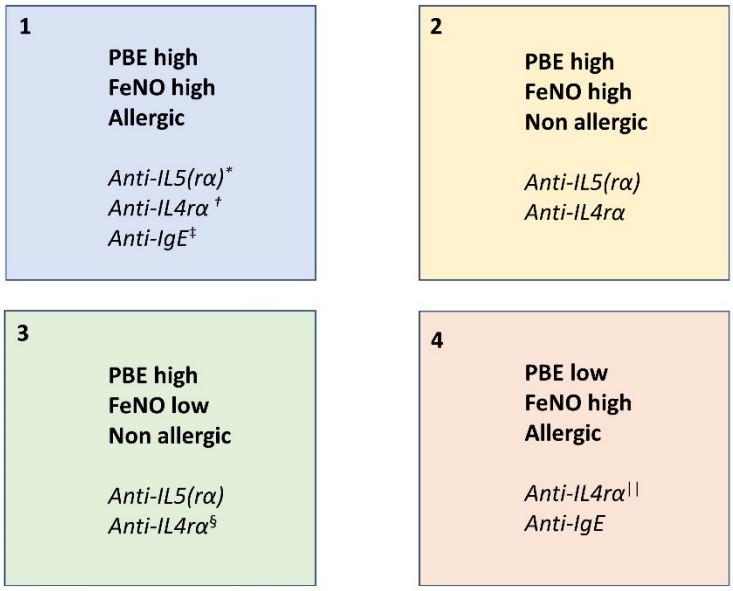

Figure 3 *Commonly occurring patterns of Type 2 inflammation in relation to choosing optimal biological therapy for severe uncontrolled asthma. Numbering corresponds to the various endotypes referred to in manuscript text. * preferred for concomitant adult-onset asthma; † preferred for concomitant chronic rhinosinusitis with nasal polyps, eosinophilic oesophagitis or concomitant atopic dermatitis; ‡ preferred for concomitant chronic idiopathic urticaria; § comparable efficacy of anti-IL5(rα) and anti-IL4rα if PBE ≥150/μL; || Anti-IL4rα preferred over anti-IgE due to greater exacerbation rate reduction. Anti-IL5(rα) preferred over anti-IL4rα for patients with endotypes 1, 2 and 3 if PBE ≥1,000/μl. PBE – peripheral blood eosinophils; FeNO – fractional exhaled nitric oxide.*

TREATING T2 COMORBIDITIES

When choosing the optimal biologic, the patient's T2 endotype should be a key driver of clinical decision making (figures 2 and 3). However, prescribers should also take pre-existing comorbidities into account as there is a potential opportunity to treat two co-related T2 conditions. For example, mepolizumab is associated with marked decreases in PBE, oesophageal eosinophilia and improved clinical outcomes in patients with eosinophilic esophagitis (EE), although it does not have a licensed indication per se.[86] Dupilumab also improves clinical outcomes in EE and reduces submucosal eosinophilia.[87] In a phase 3 RCT, dupilumab at a dose of 300mg q1w or q2w has recently been shown to improve histological outcomes, dysphagia symptoms and quality of life in patients with EE.[88] As a result of this,

dupilumab has become the first monoclonal biologic to become FDA approved for the treatment of EE. Another example would be coexistent chronic rhinosinusitis and nasal polyposis (CRSwNP) which is associated with a better anti-asthmatic response to anti-IL5[89] but does not appear to impact on nasal polyps per se at least using mepolizumab at licensed subcutaneous doses.[90] This reiterates the importance of close monitoring of patients with dual pathology and frequent liaison between different specialties in the event of a disconnected response such as improvement in asthma but not CRSwNP. Patients with CRSwNP tend to have higher PBE which probably accounts for the enhanced anti-asthmatic response to anti-IL5 in the presence of this comorbidity. Since anti-IL4rα has proven efficacy in CRSwNP[91] it seems logical to use dupilumab for patients with severe asthma especially where concomitant refractory upper airway disease is also present. If PBE is elevated above 1,000/µL along with other pertinent clinical features, then anti-myeloperoxidase and anti-proteinase-3 antibodies should be measured to refute a diagnosis of eosinophilic granulomatosis with polyangiitis (EGPA), particularly if any other clinical features are present. Higher than currently licensed doses of mepolizumab have been shown to improve disease control in EGPA,[92] and clinical trials are undergoing to evaluate benralizumab (NCT04157348).

For patients with severe T2 asthma and concomitant atopic dermatitis, anti-IL4rα is a logical option as it results in significant amelioration in disease severity and symptom burden in atopic dermatitis.[93] Finally, allergic asthmatic patients with concomitant refractory chronic idiopathic urticaria (CIU) should be trialled with anti-IgE therapy first as this has proven efficacy in both conditions.[51,94]

FURTHER CLINICAL CONSIDERATIONS

When determining T2 asthma endotype and making practical decisions on commencing biological therapies, we suggest using pragmatic FeNO and PBE thresholds of ≥25ppb and ≥300/µl respectively. Guideline recommendations for ICS-naïve patients advocate that FeNO >50ppb can be used to indicate eosinophilic inflammation and corticosteroid responsiveness.[95] Nevertheless, we feel that these cutpoints should be lower in patients taking ICS, for instance using FeNO ≥25 ppb.[96] Caution should also be exercised when interpreting FeNO levels in the presence of comorbidities. For example, one prospective study

of severe asthmatics confirmed elevated FeNO and PBE values in patients with nasal polyposis compared to to those without.[97]

For anti-IL5(rα) in the UK, NICE proposes an optimal PBE threshold of ≥300/µl in keeping with the pooled analysis from the mepolizumab and benralizumab trials[67,69] where PBE has been plotted as a continous variable for exacerbation reductions. The exception to this would be for patients who are taking maintenance OCS which markedly suppress PBE.

Recently, it has been shown that adult-onset asthma (age of diagnosis >18 years) is closely associated with disease persistence, with the presence of moderate to severe airway hyperresponsiveness and nasal polyps conferring an almost zero chance of asthma remission.[98] Another important study showed that adult-onset asthma was an important baseline factor for predicting efficacy of anti-IL5rα therapy in terms of annual exacerbation reduction and pre-bronchodilator FEV_1 improvements.[89] This was further confirmed by a real-life study demonstrating that super-responders to anti-IL5rα therapy exhibit greater baseline blood eosinophil counts, nasal polyposis and adult-onset asthma, in keeping with findings from previous phase 3 RCTs.[99,100]

In patients with raised FeNO clinicians should first of all consider treatment adherence or inhaler technique as low doses of ICS will usually suppress levels.[101,102]

A further clinical consideration is the relationship between peripheral blood and sputum eosinophil count, with more data becoming available to cast doubt on the traditionally presumed correlation.[103] A sputum eosinophil count of ≥3% is generally regarded as a raised value but in reality this has relatively little relevance in real life clinical practice as most clinicians do not perform induced sputum. Furthermore, some clinicians advocate a disconnect between peripheral blood and sputum eosinophil counts in patients with more severe asthma taking a higher ICS dose.[104] For example 1mg of inhaled fluticasone proprionate has the equivalent PBE suppressive effect as 5mg of oral prednisolone in adult asthma.[105] Preliminary data suggest that FeNO >50ppb along with PBE ≥300/µl is associated with an 80% probability of a sputum eosinophilia ≥3%.[106] In another study, FeNO was predictive of sputum eosinophilia at a cut-off point of 36ppb with a sensitivity of 67% and a specificity of 74%, whilst for blood eosinophils at a threshold of 113/µl the sensitivity was 62%

and specificity was 78%.[107] This might be important because the vast majority of asthma patients with sputum eosinophilia have mucous plugging present on HRCT.[108]

CONCLUSIONS

Ultimately the choice of biologic can be determined after careful consideration of the particular endotype, comorbidities and the existing clinical data as well as relative cost, dosing interval and availability of self injection (table 1). Our clinical experience from the MDT suggests that anti-IL5(rα) is a preferred therapeutic option for patients with SEA irrespective of FeNO or allergic status at least for patients with PBE ≥300/µl. A recent indirect treatment comparison of licensed doses showed that in asthmatic patients with similar PBE counts, mepolizumab was associated with significantly greater improvements in clinically significant exacerbations and asthma control compared to reslizumab or benralizumab,[109] however this finding was not reproduced when a matching-adjusted comparison was made.[110] There are real life data albeit preliminary to suggest that in patients who have failed on mepolizumab despite adequate PBE suppression, switching to benralizumab may be associated with improved control,[111] although it is conceivable that the same might equally apply to benralizumab failures. Efficacy of anti-IL5(rα) seems to be unrelated to FeNO levels in those patients with high PBE.

Although anti-IL4rα is most effective in patients with the high FeNO endotype, it also exhibits efficacy but to a lesser degree in patients with raised PBE and low FeNO. Until there is evidence to show that dupilumab reduces bronchial submucosal or sputum eosinophilia, we would have reservations about using it in patients with PBE ≥1,000/µl since it may also raise PBE levels. Hypereosinophilia was reported in 4.1% of patients receiving dupilumab compared to 0.6% receiving placebo.[52] Although worsening clinical symptoms were only accompanied in 0.2% of overall patients with hypereosinophilia, one potential clinical challenge clinicians face is the next treatment decision for patients with rising PBE counts but improving asthma. Hence for patients with PBE ≥1,000/µl, our MDT would suggest that until further long term safety data are available, anti-IL5(rα) seems to be the logical first line drug in such cases.

The best evidence for OCS sparing is with using anti-IL5(rα) or anti-IL4rα rather than anti-IgE. Since anti-IL4rα suppresses IgE levels as well as FeNO we would advocate this over anti-IgE in

patients with the FeNO-high, allergic endotype regardless of PBE status, especially as the magnitude of exacerbation reduction seems to be more impressive. Likewise, we would suggest using anti-IL5(rα) as first line rather than anti-IgE in patients with the PBE-high, allergic endotype irrespective of FeNO due to a greater reduction in exacerbations seen with the former.

Ultimately head-to-head trials are urgently required to compare the different biologics across common type 2 endotypes, such as the PREDICTUMAB trial (NCT03476109) comparing mepolizumab and omalizumab. We also look forward to more data becoming available on tezepelumab [NCT03927157], a monoclonal antibody directed against thymic stromal lymphopoietin, which has shown promising exacerbation reductions in phase 2.[112] Since tezepelumab blocks signalling of the IL4, IL5 and IL13 pathways and suppresses PBE, FeNO and IgE, one might consider this to be the most broad spectrum of current biologics.

Targeting downstream type 2 cytokines or upstream epithelial alarmins for severe asthma

ABSTRACT

Biologics, including omalizumab, mepolizumab, benralizumab, and dupilumab, targeting downstream IgE, cytokines IL-5, and IL-4/13, respectively, have shown promising effects in terms of reduction in annualized asthma exacerbation rates (AER), oral corticosteroid-sparing effects, improvements in forced expiratory volume in 1 second, and improved Asthma Control Questionnaire scores. However, despite these welcome advances, approximately 30% of patients with severe asthma receiving biologics tailored to their specific downstream type 2 biomarkers, including total IgE, peripheral blood eosinophils, and fractional exhaled nitric oxide, do not experience meaningful improvements in their AER. Instead of blocking downstream cytokines, targeting upstream epithelial alarmins, including IL-33, thymic stromal lymphopoietin, and IL-25, has been proposed to tackle the immunologic heterogeneity of asthma. This review article aims to pragmatically summarize the latest key clinical data on anti-alarmin therapies in severe asthma and put these findings into context with regard to currently available downstream cytokine blockers.

INTRODUCTION

In recent years, new therapeutic options have become available for patients with refractory severe asthma driven by type 2 (T2) inflammation.[19] Biologics including omalizumab, mepolizumab, benralizumab and dupilumab targeting downstream immunoglobulin E (IgE), cytokines IL-5 and IL-4/13 respectively have shown promising effects in terms of reduction in annualised asthma exacerbation rates (AER); oral corticosteroid (OCS) sparing effects; improvements in forced expiratory volume in 1 second (FEV_1) and asthma control questionnaire (ACQ).[1]

However, despite these welcome advances, it is increasingly recognised that approximately 30% of severe asthma patients receiving biologics tailored to their specific downstream T2 biomarkers including total IgE, peripheral blood eosinophils (PBE) and fractional exhaled nitric oxide (FeNO) do not experience meaningful improvements in their AER.[1] Super-responders to

biologics are characterised as having no exacerbations and cessation of maintenance OCS accompanied by a large improvement in asthma control, comprising two or more times the minimal clinically important difference (MCID),[113,114] although in real life such cases are the exception. In contrast, a study of 250 moderate-to-severe asthma patients receiving mepolizumab or reslizumab therapy revealed that 43% experienced a suboptimal treatment response with the latter being associated with daily OCS requirement, sinus disease and late onset asthma.[76] Hence there clearly remains an unmet need for many patients taking current biologics as monotherapy. Furthermore, whilst improvements in FEV_1 and ACQ are statistically significant in multicentre randomised controlled trials (RCTs), we should interpret these findings in the context that they do not exceed the MCID of 230 ml and 0.5 units respectively.[64,115]

Instead of blocking downstream cytokines, targeting upstream epithelial alarmins including IL-33, thymic stromal lymphopoietin (TSLP) and IL-25 has been proposed to tackle the immunologic heterogeneity of asthma.[116] This review article aims to pragmatically summarise the latest key clinical data on anti-alarmin therapies in severe asthma and put these findings into context with regards to currently available downstream cytokine blockers (Table 2). It is not meant to be an exhaustive systematic review nor will the aim be to discuss current available downstream anti-cytokine therapies in detail as it has previously been published.[1] Furthermore, the scope of this review will not include a detailed discussion on omalizumab.

Table 2 *RCT data on effects of anti-alarmin therapy and downstream cytokine blockade on pulmonary function, asthma control, annualised exacerbation rate, type 2 biomarkers and airway hyperresponsiveness for severe asthma patients compared to placebo*

	Anti-IL33	Anti-TSLP	Anti-IL4rα	Anti-IL5(rα)
FEV_1 (L)	↑	↑	↑	↑
FEF_{25-75} (L/s)	↑	N/A	↑	N/A
ACQ	↓	↓	↓	↓
AER	N/A	↓	↓	↓
PBE (cells/µl)	↓	↓	↑/↔	↓↓
FeNO (ppb)	↓	↓	↓	↔
Total IgE (IU/ml)	↓	↓	↓	↔
OCS sparing	N/A	↔	↓	↓
AHR	N/A	↓	N/A	N/A

ACQ = asthma control questionnaire; AER = annualised exacerbation rate; AHR = airway hyperresponsiveness; FeNO = fractional exhaled nitric oxide; FEF_{25-75} = forced expiratory flow rate between 25 and 75% of full vital capacity; FEV_1 = forced expiratory volume in 1 second; IgE = immunoglobulin E; OCS = oral corticosteroid; PBE = peripheral blood eosinophils; TSLP = thymic stromal lympoietin

DOWNSTREAM TARGETING OF IL-4/5/13 PATHWAYS

In the United Kingdom, Europe and USA commonly used current licensed subcutaneously administered biologics which block downstream cytokines for the treatment of severe asthma include mepolizumab, benralizumab and dupilumab, with intravenous reslizumab being reserved for patients with higher body mass.[117-120] The National Institute for Health and Care Excellence and the Scottish Medicines Consortium have recently approved dupilumab only as second line for those who have previously failed on anti-IgE or anti-IL5 therapies.[121,122] We have previously published a pragmatic review article examining factors that determine optimal choice of biologic therapy for patients including disease endotype, patient preference and presence of concomitant type 2 comorbidities.[1] All classes of biologics targeting IL5 and IL4/13 pathways have been shown in systematic and Cochrane reviews to improve exacerbation rates by approximately 60-70% as well as OCS sparing effects amounting to approximately a 50% dose reduction.[63,123,124] Figure 4 depicts their effects on commonly measured T2 biomarkers in clinical practice. These main classes of cytokine blockers have also been shown to significantly improve FEV_1 and ACQ although these do usually not exceed MCID

aside from super-responders.[63,71] In the phase 3 RCT involving benralizumab, the median difference reduction in OCS dose amounted to 50% compared to placebo, whilst for mepolizumab after 24 weeks there was a 50% median reduction in OCS dose.[125,126] Comparatively the phase 3 trial studying dupilumab showed a slightly lesser OCS sparing effect after 24 weeks with a 28% difference.[127] Another open-labelled real life study (PONENTE) with benralizumab showed that 63% of patients were able to wean off OCS completely.[128]

TEZEPELUMAB (ANTI-TSLP)

TSLP is a key epithelial alarmin involved in binding of antigen presenting cells in turn resulting in activation of downstream type 2 inflammatory cytokines including IL-4, IL-5 and IL-13 (Figure 4).[129-131] In addition TSLP is involved in interactions between airway epithelium and other immune cells which are not part of the type 2 inflammatory process per se.[130] In allergic eosinophilic asthma, TSLP initiates pathways involving TH2 lymphocytes, basophils and mast cells to generate airway eosinophilia. TSLP can also directly stimulate mast cells to produce T2 cytokines, whilst mast cells can produce significant amounts of TSLP from IgE cross-linking.[130]

Tezepelumab is a monoclonal antibody (IgG2λ) which specifically binds to the TSLP ligand in turn blocking receptor activation. In the phase 2b PATHWAY trial over 52 weeks, tezepelumab 210mg every 4 weeks reduced the primary end point of overall AER by 71% (90%CI 54,82).[112] Here significant reductions in AER were evident in patients with both type 2 low and high disease using a threshold of PBE ≥250 or <250 cells/µl or FeNO <24 or ≥24ppb. Furthermore, tezepelumab conferred reductions in PBE, FeNO and total IgE compared to placebo inferring a broad-spectrum effect by attenuating downstream cytokine signalling including IL-4 (IgE), IL-13 (FeNO) and IL-5 (PBE) (figure 4). However, reductions in PBE amounting to a mean fall of approximately 150 cells/µl from a mean baseline of 365 cells/µl are not as profound as those seen with anti-IL5 agents.

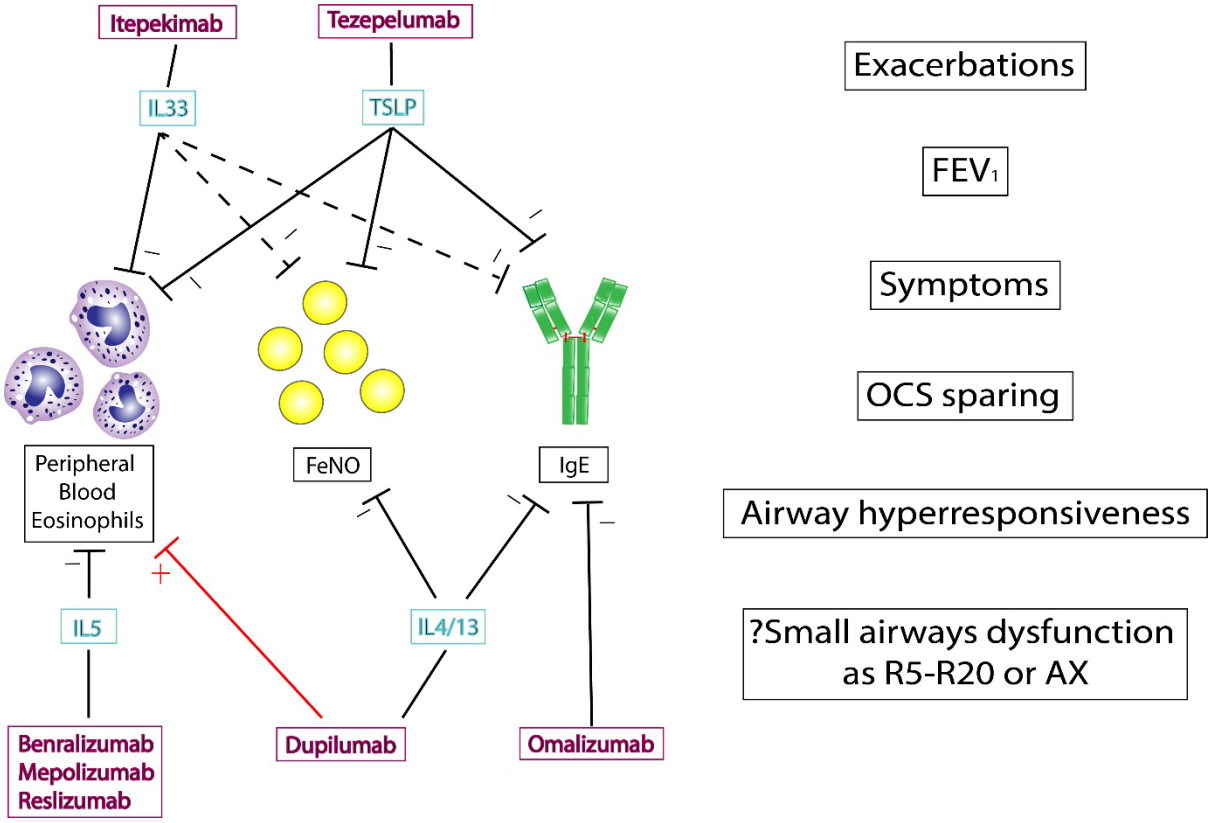

Figure 4 *Effects of biologic and anti-alarmin therapies on upstream epithelial alarmins, downstream cytokines and type 2 biomarkers in the context of key patient clinical outcome measures in severe asthma. Hyper-eosinophilia may be associated with dupilumab whilst only tezepelumab has been shown to improve airway hyperresponsiveness. All biologics significantly improve ACQ and prebronchodilator FEV1 and reduce OCS requiring exacerbations. Interrupted line refers to small but significant suppressive effect of itepekimab on FeNO and total IgE.*

In the subsequent phase 3 NAVIGATOR trial over 52 weeks, tezepelumab 210mg significantly improved the primary end point resulting in an overall 56% (95%CI 47,63) reduction in AER. Tezepelumab also conferred significant mean improvements in key secondary end points including FEV_1 (130 ml), ACQ (-0.33) and AQLQ (-0.34), although these were all less than their respective MCIDs.[132] As in the PATHWAY trial there were decreased T2 biomarkers with mean falls amounting to 14ppb in FeNO; 130 cells/µl in PBE and 208 IU/ml in total IgE compared to placebo.

Of note, post hoc analysis of the primary end point in NAVIGATOR showed that AERs were significantly reduced in the tezepelumab group to a greater degree in patients who had higher eosinophil counts.[132] For example, there was a 70% relative reduction in AER (95%CI 60,78) associated with baseline PBE ≥300 cells/µl compared to 41% reduction (95%CI 25,54) with

<300 cells/µl, which is a significant difference as indicated by CIs which do not overlap. Even in patients with PBE <150 cells/µl there was a 39% (95%CI 12,68) reduction in AER. The same was observed in regard to non-overlapping CIs for AERs with 68% (95%CI 58,75) reduction for FeNO ≥25ppb versus 32% (95%CI 8, 49) for FeNO <25ppb. For patients with type 2 low asthma who had both PBE <300 cells/µl and FeNO <25ppb there were borderline significant reductions in AER compared to placebo amounting to 29% (CI 0,50). Intriguingly, for patients with PBE ≥300 cells/µl and FeNO <25ppb a 39% reduction in AER was observed (95%CI -7,65) although this was non-significant which could be related to lower patient numbers in this particular subgroup analysis. The greatest reduction in AER was seen in those patients who had type 2 high asthma with PBE ≥300/µl and FeNO ≥25ppb where there was a 77% (95%CI 67,84) reduction. The wide CIs for type 2 low patients indicates that in such cases there is considerable heterogeneity in response to tezepelumab, as compared to the much narrower CIs in type 2 patients with a more homogenous response. In other words, clinicians can expect a more predictable response to tezepelumab in those individuals with type 2 high asthma.

Tezepelumab improves AER in patients with type 2 low asthma potentially via a separate immunological pathway from the traditional IL4/5/13 paradigm. A previous study showed that TSLP stimulates dendritic cells to release IL6 and IL23 that contribute to the maturation of naïve CD4+ cells to T helper 17 lymphocytes.[133] Similarly, another study found that TSLP has the capability of stimulating T helper 2 and 17 responses simultaneously, increasing expression of downstream cytokines such as IL4 and IL17A respectively.[134]

When inspecting data for AER in NAVIGATOR where type 2 biomarkers were plotted as a continuous variable it is evident that the slope is much steeper for increased AER with placebo compared to reduced AER with tezepelumab, this being the case for both PBE and FeNO. In contrast the separation between regression lines remains constant across the range for total IgE, indicating that this is not a key determinant of response. For FEV_1 the response was greater among those with PBE ≥300 cells/µl: 230ml (95%CI150, 310) compared to PBE <150 cells/µl: 30ml (95%CI -70,130), with non-overlapping CIs indicating a significant difference. Similar results occurred for ACQ: -0.50 (95%CI -0.69, -0.31) versus -0.09 (-0.33, 0.16).

Taken together these results from phase 2/3 trials suggest that blocking the upstream alarmin TSLP with tezepelumab results in clinically meaningful improvements in asthma control in patients with type 2 high asthma with regard to exacerbations, ACQ and FEV_1. Tezepelumab

appears to also confer lesser degrees of improvements in type 2 low asthma in relation to exacerbation reductions but not for FEV_1 or ACQ, along with a more variable response. Nonetheless, it is notable that tezepelumab is the first biologic with at least some degree of activity in type 2 low refractory severe asthma which is at present an unmet need. It would be helpful to have type 2 low biomarkers which might be able to predict a better response with tezepelumab in preventing exacerbations.

Preliminary abstracted data from the phase 3 SOURCE trial[135] (NCT03406078) with subcutaneous tezepelumab 210mg over 48 weeks in severe OCS dependent asthma patients were disappointing in terms of showing an overall non-significant 22% (CI -47,31) reduction in the primary end point of OCS dose along with no signification reduction in AER: 31% (95%CI -9,56). One plausible explanation for this result was related to the large OCS sparing response in the placebo arm amounting to 46% of patients experiencing a 90-100% reduction in OCS dose. This in turn potentially provides some insight into our current clinical practice where we are perhaps not proactively weaning our patients off maintenance OCS at routine appointments. Alternatively, this could in part also be explained by improved adherence to ICS therapy in the clinical trial setting. Nevertheless, post hoc analysis in patients with baseline PBE ≥300/µl revealed 71% (CI 14,90) OCS dose reduction, with the wide CI indicating a variable response perhaps due to the inherent PBE suppressive effect of OCS. Post hoc analysis of OCS dependent patients in NAVIGATOR observed 28% (95%CI -26,59) reduction in AER indicating futility for Tezepelumab, although in such patients there were improvements in FEV_1 of 270ml (95%CI 100,440) and ACQ of -0.65 (95%CI -1.08,-0.22), both of which exceeded MCIDs of 230ml and 0.5 units.[136] Given the impressive overall results of NAVIGATOR and the known OCS sparing effect of blocking downstream IL4/13 signalling with dupilumab,[127] it is difficult to explain this anomaly with tezepelumab. A potential explanation for this phenomenon has been proposed as a two-compartment model for type 2 inflammation recently.[137] It is hypothesised that for a biologic to be OCS sparing, it must effectively regulate the systemic compartment of circulating eosinophils (anti-IL5rα) or prevent eosinophils from escaping the vascular compartment (anti-IL4rα). Tezepelumab reduces airway chemotactic pull mediated by IL-13 and measured using FeNO by a similar magnitude to dupilumab (Table 3) albeit with suboptimal eosinophil suppression compared to anti-IL5rα. Nonetheless both tezepelumab

and dupilumab appear to confer similar clinical impacts on asthma control as AER, ACQ and improved lung function as FEV_1 (Table 3).

Table 3 *Mean Improvements in AER, FEV_1, FeNO, and asthma control with tezepelumab or dupilumab versus placebo from phase 3 trials*

		Dupilumab Liberty Quest[52]	**Tezepelumab Navigator[132]**
AER	Baseline	2.09	2.11
	Absolute Δ	0.98	1.18
	% Δ	-47%	-56%
FEV_1 (L)	Baseline	1.78	1.85
	Absolute Δ	0.17	0.13
	% Δ	9.6%	7.0%
FeNO (ppb)	Baseline	35	44
	Absolute Δ	-12.3	-13.8
	% Δ	-35.1%	-31.4%
ACQ	Baseline	2.8	2.8
	Absolute Δ	-0.31	-0.33
	% Δ	-11.1%	-11.8%

Another key part of the asthma disease phenotype is the presence of airway hyperreactivity (AHR) which can be measured by indirect bronchial challenge using the osmotic agent mannitol. Ex vivo it has been shown that IL-13 is a key cytokine in mediating AHR which can be blocked by dupilumab.[138] The CASCADE phase 2 RCT in uncontrolled asthma investigating tezepelumab 210mg Q4W demonstrated a significant (p=0.03) 1.15 doubling dose improvement in the secondary end point of mannitol AHR compared to placebo as well as significantly reducing the primary end point of airway biopsy eosinophils.[129] Meanwhile the UPSTREAM phase 2 RCT in patients with uncontrolled asthma using intravenous tezepelumab 700mg found a mean 0.9 doubling dose difference in the primary outcome of mannitol AHR which was not significant (p=0.06), while airway biopsy and lavage eosinophils were both significantly suppressed.[139]

Tezepelumab is therefore unique amongst the current available biologics in the sense that it significantly suppresses all three type 2 biomarkers (PBE, total IgE and FeNO) as well as attenuating AHR (figure 4). The lack of apparent efficacy in OCS dependent patients requires further investigation given the known OCS sparing efficacy of anti-IL5 and anti-IL4rα agents.[125-127] In a sense tezepelumab could be considered to have similar efficacy to

dupilumab in terms of IL-4/13 blockade through FeNO and IgE suppression but with the additional action of IL-5 blockade partially suppressing blood eosinophils. Thus, tezepelumab confers a theoretical advantage over dupilumab in terms of obviating escape of blood eosinophils.

We are intrigued to know if locally acting inhaled anti-TSLP will prove to be as effective as systemic tezepelumab given that the former may not adequately address the systemic component of type 2 inflammation or indeed be able to target the small airways. In a recent RCT, 12 weeks of therapy with the potent inhaled anti-TSLP CSJ117 was shown to reduce allergen induced bronchoconstriction, sputum eosinophilia and FeNO levels in mild allergic asthma patients compared to placebo.[140] The putative difference between inhaled and injected anti-TSLP is analogous to patients with refractory severe asthma despite using high dose ICS who can then be adequately controlled by a small maintenance dose of OCS inferring a systemic component to refractory type 2 inflammation.

ASTEGOLIMAB (ANTI-ST2) AND ITEPEKIMAB (ANTI-IL33)

IL-33 is an inducer of TH2 innate and adaptive immunity and signals via the IL-1 receptor related protein ST2 triggering the release of chemokines and cytokines that promote T2 inflammation.[141] Elevated levels of IL-33 mRNA produced by airway smooth muscle cells are detected from biopsies of asthma patients compared to control subjects, especially those with severe asthma.[142] Along with IL-5, IL-33 is involved in the production, activation and survival of eosinophils and hence plays a key role in T2 high asthma.[143] Interestingly, dexamethasone fails to significantly dampen down TNFα-generated upregulation of IL-33 ex vivo.[142]

In a phase 2b RCT of patients with uncontrolled severe asthma,[144] the anti-ST2 monoclonal antibody astegolimab met the primary endpoint of AER reduction over placebo at 490mg every 4 weeks, alongside a significant increase in duration to first asthma exacerbation and improvements in asthma quality of life. Although somewhat limited by sample size in the subgroup analysis, there were no observed reductions in AER over placebo in patients with PBE ≥300 cells/µl. On the contrary, patients with PBE <300 cells/µl experienced a significant

54% (95%CI 25,71) AER reduction over placebo suggesting promising preliminary results in type 2 low asthma.

In a phase 2 RCT the anti-IL33 monoclonal antibody itepekimab at a subcutaneous dose of 300mg every 2 weeks was given alone, dupilumab 300mg alone, combination therapy or placebo were evaluated in patients initially maintained on ICS/LABA for the first four weeks, with LABA discontinued at week 4 and ICS tapered over 2-3 weeks starting at week 6. For the primary outcome of loss of asthma control after 12 weeks, itepekimab alone was associated with 58% (95%CI 12,80) reduction compared to placebo and dupilumab alone was associated with a 67% (95%CI 30,85) reduction.[145] The combination of itepekimab and dupilumab had a 48% (95%CI -6,74) reduction which was not significant versus placebo and was no better than either drug alone. For secondary end points, mean improvements in FEV_1 of 0.14L (95%CI 0.01,0.27) were seen with itepekimab alone and 0.16L (95%CI 0.03,0.29) with dupilumab alone which were both significant compared with placebo but less than MCID, while the combination was no better than placebo.[115] Improvements in ACQ were all statistically significant versus placebo for mono and combination therapy but again were less than the MCID. Interestingly, PBE increased with dupilumab alone but not with combination or itepekimab alone suggesting itepekimab blocks downstream signalling of IL-5. Although improvements in control were generally greater in patients treated with dupilumab than with itepekimab especially in those with T2 high asthma, the study was not powered to detect such differences. Itepekimab alone reduced FeNO and IgE compared to placebo inferring interruption of IL-13 or IL-4 signalling although not to the same degree as the combination. In patients with PBE <300 cells/µl no significant impact was seen on asthma control or FEV_1 with any of the randomised treatments, albeit with small patient numbers, in turn suggesting that itepekimab has no effect in type 2 low disease.

BRODALUMAB (ANTI-IL25)

IL-25, also known as IL-17E, is produced by bronchial epithelial cells and activates TH2 cells, basophils, eosinophils and mast cells thus perpetuating the T2 inflammation response in asthma.[146,147] It has been shown ex vivo that IL-25 is associated with angiogenesis and airway remodelling, both of which contribute to asthma severity.[148] IL-25 binds to the heterodimeric receptor complex composed of IL-17RA and IL-17RB.[149] TH17 cells predominantly exert their

action by producing the IL-17 family of cytokines (IL-17A–17F), of which IL-17A and IL-25 (IL-17E) are thought to play pivotal roles in pulmonary inflammation through IL-17RA-containing heterodimeric receptor complexes expressed in airway smooth muscle cells.[149]

In a phase 2a RCT studying the anti-IL17RA monoclonal antibody brodalumab, which blocks IL-17A, IL-17F and IL-17E (IL-25), no improvements were observed in the primary endpoint of ACQ after 12 weeks of treatment in patients with inadequately controlled moderate to severe asthma.[150] Any improvements in the secondary endpoint of FEV_1 also did not amount to statistical significance or a clinically meaningful response. In a subgroup analysis of patients with a mean bronchodilator response of 33% in the same study,[150] a significant ACQ improvement that exceeded MCID was shown with brodalumab 210mg every 2 weeks. These findings are perhaps not entirely surprising due to the known complexity and heterogeneity in asthma pathophysiology. Consequently, further RCTs are required to assess the efficacy of anti-IL25 blockade on other key outcomes such AER and OCS dose reduction and its effect on FeNO, PBE and IgE.

PROPOSED BIOLOGIC AND ANTI-ALARMIN FLOWCHART

Based on specific disease endotypes and current best available evidence, figure 5 represents the type 2 biomarker pivot in relation to FeNO and PBE and proposes a putative pragmatic clinical flowchart recommending optimal first- and second-line downstream cytokine blocker or upstream epithelial anti-alarmin options for the management of patients with severe refractory asthma. Of note, this flowchart refers to patients who are presently not taking oral corticosteroids and is irrespective of allergic status. Thresholds of <300, 300-1000, and ≥1000 cells/µl are utilised to denote low, medium and high peripheral blood eosinophil counts respectively. The arbitrary cut-off of 1000 cells/µl was determined based on our clinical experience and that of others.[151] Indeed, in our Tayside specialist asthma clinic, a peripheral blood eosinophil count ≥1000 cells/µl would usually mandate testing for myeloperoxidase and proteinase 3 antibodies which if positive would suggest eosinophilic granulomatosis with polyangiitis.[152] Similarly, thresholds of <25, ≥25ppb and ≥50ppb were used to represent low, medium and high levels of FeNO according to current American Thoracic Society guidelines.[153]

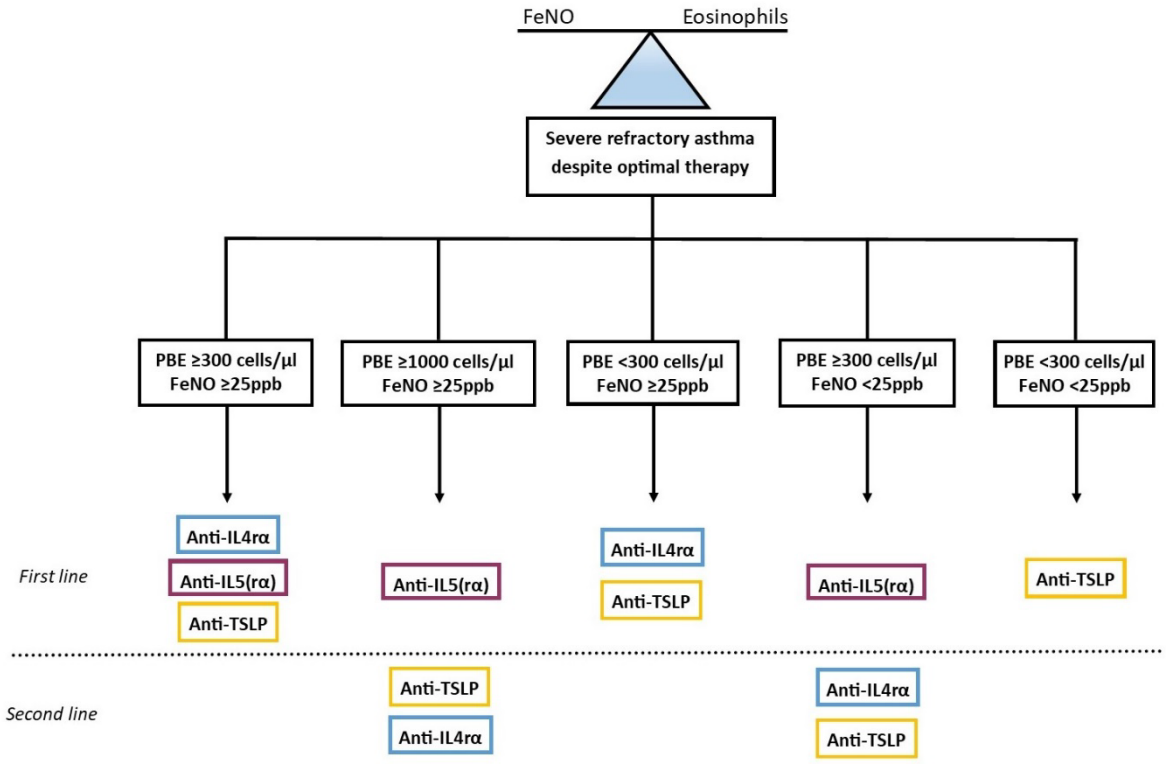

Figure 5 *Type 2 biomarker pivot in regard to FeNO and blood eosinophils depicting the proposed clinical flowchart in severe refractory asthma despite optimal inhaled and oral therapies. Putative recommendations on first- and second-line biologics are shown according to specific disease endotypes irrespective of allergy status in patients not taking oral corticosteroids. In patients who have peripheral blood eosinophil counts ≥300 cells/µl and FeNO ≥25ppb, theoretically either anti-IL4rα, anti-IL5(rα) or anti-TSLP could be considered first line, although in patients with FeNO ≥50ppb, indicating persistent IL-13 escape, anti-IL5(rα) might become relatively less effective. FeNO ≥50ppb would in turn predict a better response to anti-IL4rα or anti-TSLP. Anti-IL4rα may occasionally be associated with hyper-eosinophilia especially in patients with baseline PBE ≥1000 cells/µl, while anti-TSLP suppresses PBE albeit to a lesser degree than anti-IL5(rα).*

The recommendations stratified by high, medium, and low levels of PBE and FeNO are based on the following considerations. Firstly, in patients with moderate PBE together with moderate or high FeNO, either anti-IL5(rα) or anti-IL4rα could potentially be used as first line as both have been shown to be effective in T2 high disease.[1] Secondly, those patients with high PBE (irrespective of FeNO) have predominantly IL-5 driven disease and therefore should receive anti-IL5(rα) as first line.[73] In a real life setting the clinical effectiveness of mepolizumab and benralizumab was independent of baseline FeNO level in severe eosinophilic asthma.[81] Whilst dupilumab has been shown to be more effective in those with either PBE ≥300 cells/µl

or FeNO ≥50ppb[52] there is a putative concern regarding the risk of inducing hypereosinophilia due to IL-5 escape and therefore some caution should perhaps be exercised when prescribing this in patients with pre-existing hyper-eosinophilia or that which could be masked by prior anti-IL5 or OCS treatment. Having said that it remains unclear if such escape of PBE is clinically relevant if as suggested blocking IL-13 signalling results in suppression of tissue eosinophils.[79] Thirdly, patients with low PBE and raised FeNO have predominantly IL-13 driven disease and therefore anti-IL4rα would be considered first choice. Lastly, similar to the second group, those patients with medium PBE and low FeNO should initially be trialled on anti-IL5(rα) therapy. Although dupilumab is more effective in patients with PBE ≥300 cells/µl, one might perhaps consider using it as second line in this group due to low FeNO.

Where might anti-TSLP fit into this pathway given its broad-spectrum effects on inhibiting downstream signalling of IL-4/5/13? One possibility is that tezepelumab could be used wherever dupilumab is indicated especially in patients with high FeNO given that it exhibits similar inhibitory effects on downstream IL-4/13 signalling. However, as tezepelumab appears to obviate potential concomitant eosinophil escape it might be preferable to dupilumab in those patients with high PBE. Notably, we would not advocate tezepelumab as a first line alternative to anti-IL5 in patients with high PBE and low FeNO as it appears to only attenuate downstream IL-5 signalling. Tezepelumab is presently also the only biologic to work in T2 low disease and therefore would be the optimal choice for such patients, albeit bearing in mind the likelihood of a more variable response.

Although we appreciate that figure 5 is somewhat speculative in the absence of head-to-head trials, this is based on clinical experience and current best available evidence. Our current Scottish guidelines support severe asthma patients with PBE ≥150 cells/µl receiving anti-IL5 therapy[154] although we would personally only consider ≥300 cells/µl as truly eosinophilic while at the same time predictive of a more meaningful clinical response.[69] One can also debate whether flowcharts offering three or four first-line options may be of questionable use to the practising clinician. In a sense it is rather intellectually naive to believe that a single biologic can be used to achieve optimal control aside from occasional super-responders. In real life use of biologic combinations is likely to be prohibitive from a cost perspective until further data from RCTs becomes available. Ultimately, head-to-head trials are required to

answer the important question of which anti-alarmin or combination of biologics is best suited to which patient.

FURTHER CLINICAL CONSIDERATIONS

In phase 3 trials of biologics including anti-alarmins, all drugs have been shown to significantly reduce exacerbations and improve FEV_1. While downstream cytokine blockers have OCS sparing effects thus far anti-TSLP does not. Downstream cytokine blockers and anti-TSLP are all more effective in type 2 high patients with raised PBE, while drugs which block IL13 signalling including dupilumab and tezepelumab are more effective in patients with raised FeNO. Due to the unlikelihood of head-to-head biologic trials in the near future, we performed a brief indirect treatment comparison of dupilumab versus tezepelumab using phase 3 clinical trial data[52,132] with regards to lung function, asthma control and FeNO (Table 3). Absolute percentage improvements in FEV_1, FeNO, ACQ and AER were largely comparable for either therapy calculated from a similar baseline. Interestingly, greater percentage improvements in total IgE were observed with dupilumab than with tezepelumab, possibly due to more targeted downstream blockade of IL-4 and IL-13. However, we appreciate such comparisons, although intriguing, are often crude and should perhaps be interpreted with some caution.

Improvements in ACQ are significant across all biologics including anti-alarmins and although questionnaires are subjective and influenced by patient expectations, perceptions and comorbidities, their inclusion is vital for patient-and-clinician and interdisciplinary shared decision-making.

It is known that small airways dysfunction (SAD) is associated with poor asthma control, a higher asthma exacerbation rate and more severe airway hyperresponsiveness.[155,156] However, the effect of biologic therapy on SAD has not been well characterised. Itepekimab has been shown to significantly improve forced mid expiratory flow rate (FEF_{25-75}) by 0.17L/s (95%CI 0.01, 0.33) compared to placebo in patients with severe asthma whilst in the same study dupilumab improved FEF_{25-75} by 0.19L/s (95%CI 0.03, 0.35) compared to placebo.[145] However, these improvements did not exceed the biological variability value of 0.21L/s for FEF_{25-75} in severe asthma.[157] One real-life retrospective study showed that biologic therapy

with mepolizumab or omalizumab improves FEF_{25-75} in severe asthmatics and that the difference in impulse oscillometry as resistance heterogeneity between 5 and 20Hz (R5-R20) also significantly improved with biologic therapy in those with baseline R5-R20≥0.08kPa/L/s,[158] in conjunction with significant improvements in asthma exacerbations and ACQ.[159] Another real-life study revealed significant improvements in FEF_{25-75} after 24 weeks of benralizumab therapy in allergic patients with severe eosinophilic asthma.[160] Clearly further prospective placebo-controlled studies powered a priori in patients who exhibit abnormal small airways function at baseline are required, powered on outcomes such as respiratory oscillometry or perhaps nitrogen washout.

There is emerging interest in the effect of biologic therapy on type 2 (T2) comorbidities including chronic rhinosinusitis with nasal polyps (CRSwNP), eosinophilic oesophagitis, chronic idiopathic urticaria (CIU) and atopic dermatitis.[91,161-163] One perhaps would expect a so-called higher burden of T2 inflammation in patients with concomitant T2 comorbidities. One retrospective study showed that patients with concomitant moderate-to-severe asthma with CRSwNP had higher circulating levels of FeNO and PBE compared to those with asthma alone.[3] Benralizumab is more effective in patients with asthma and CRSwNP than patients with asthma alone although this might be due to the presence of higher PBE in such patients.[69] All biologics currently used for the treatment of asthma are efficacious in CRSwNP although at present when using indirect treatment comparisons dupilumab seems to be the most effective.[164,165] Studies demonstrating similar associations with other combinations of T2 conditions would be of interest as further characterisation of the disease endotype could help clinicians choose optimal biologic therapy. We look forward to the results of large ongoing clinical trials investigating the effects of tezepelumab on CRSwNP (NCT04851964) and CIU (NCT04833855).

Currently there is an unfortunate lack of trial data concerning head-to-head biologic comparisons, but we anticipate the results of the PREDICTUMAB trial (NCT03476109) studying the magnitude and prediction of omalizumab versus mepolizumab response in adult patients with severe asthma.

Another pertinent area is the identification of factors that separate biologic super-responders from suboptimal- or non-responders. For example, in large RCTs, the odds ratios (95% CI) for mepolizumab and benralizumab to reduce OCS requirement by 100% were 1.67 (0.49,5.75)

and 4.19 (1.58,11.12) respectively.[125,126] We would especially like to see RCTs looking at combination therapy on AER, for example comparing benralizumab plus dupilumab (i.e. co-benradupilumab) versus respective monotherapy in patients with both high PBE and FeNO who are not controlled on either drug alone, to examine whether blocking all three downstream cytokines together confers additivity of response. This would test the hypothesis as to whether eosinophil escape via IL-5 or FeNO escape via IL-13 is pertinent in regards to achieving optimal control. For example, in our severe asthma clinic we often see patients who reduce their AER from say 6 to 2 on antiI-IL5 in the presence of eosinophil suppression but persistent FeNO elevation. Obviously the cost of such combination therapy would in many countries be prohibitive although this might be counterbalanced by improved quality of life and in particular time off work. Although TSLP blockade might conceivably achieve attenuated signalling of IL-4/5/13 it should be borne in mind that eosinophil suppression with tezepelumab is much less effective compared to anti-IL5. Hence for patients with high PBE ≥1000 cells/µl one might prefer to start off using anti-IL5. Moreover, there is a paucity of data regarding the effects of biologics on AHR except for tezepelumab. Therefore, we look forward to the results with benralizumab (EudraCT number 2019-003763-22) and dupilumab (EudraCT number 2021-005593-25) powered on mannitol AHR in patients with uncontrolled severe asthma.

Hyper-eosinophilia with systemic manifestations is a potential concern when commencing patients on dupilumab therapy as reflected by the manufacturer's prescribing information.[151,166] In rare circumstances, anti-IL4rα therapy has been associated with sudden deterioration of asthma, eosinophilic tissue infiltration and eosinophilic granulomatosis with polyangiitis-like symptoms.[167] This is especially precarious if pre-existing hyper-eosinophilia has been masked beforehand by oral corticosteroids or anti-IL5 therapy and this therefore would be a further argument for considering combined dupilumab and benralizumab or indeed tezepelumab alone for such patients. We are particularly intrigued to find out whether the observed improvements in AER, FEV_1 and ACQ with current monotherapy phase 3 data would be additive for combination biologic therapy. Although one might expect this to be the case, we are reminded that phase 3 data looking at combination therapy with the eosinophil suppressor itepekimab along with dupilumab was not superior to either drug alone compared to placebo in regards to loss of asthma control, FEV_1 or ACQ.[145]

More data are required on airway tissue biopsy and sputum cells especially eosinophils given the preliminary negative results of the EXPEDITION trial (NCT02573233) showing no impact of dupilumab versus placebo on airway inflammatory cells, especially as the UPSTREAM trial with tezepelumab showed reduction of airway eosinophils. In this regard, benralizumab has also been shown in a phase 1 RCT to significantly reduce airway mucosal/submucosal eosinophils.[168]

In conclusion, although the underlying disease endotype is undoubtedly a crucial part of the immunological puzzle of asthma that requires solving, there are likely other currently unknown factors also at play. For example, if eosinophilic inflammation is the main driver of asthma exacerbations, can eosinophil depletion rather than suppression fully explain why switching from anti-IL5 to anti-IL5rα therapy significantly improve FEV_1, asthma control and OCS dose requirement in sub-optimal responders to the former?[111,169] Although head-to-head biologic trials are unlikely in the near future, we would be especially keen to see key patient outcomes along with safety data in response to combination biologic therapy.

Chapter 2: Methods

Asthmatic participant selection

Participants with severe asthma were directly recruited into the BISA trial from the respiratory clinics held in NHS Tayside. Patients were invited to attend for a screening visit based on their likely suitability for study entry dependent on their recorded data that included: age, asthma severity, onset of asthma symptoms, lung function, asthma control questionnaire, current treatment regimen and peripheral blood eosinophil count. Patients were in receipt of a written participant information sheet that provided details of specific study involvement and requirements, before attending for screening. All participants were given the opportunity to ask questions and provided written informed consent in my presence.

All patients included in these studies were aged between 18 and 75 years upon study entry. Furthermore, they all had a confirmed respiratory physician-based clinical diagnosis of severe asthma according to current guidelines. Patients were required to go through a screening process for their safety prior to study entry that demonstrated a normal physical examination by a doctor, urinalysis to exclude pregnancy if applicable, and screening blood tests comprising full blood count and specific IgE. The details of the specific study entry criteria are provided within the individual study.

All study protocols, patient information sheets, informed consent forms and other study materials were scrutinized and approved by the East of Scotland Research Ethics Committee. Furthermore, for clinical trials of investigational medicinal products, further review and approval was gained from the Medicines and Healthcare products Regulatory Authority (MHRA) prior to study commencement.

In relation to the retrospective studies in this thesis, a secure password protected database was created to include all moderate to severe asthma patients who had previously attended the Scottish Centre for Respiratory Research (SCRR) for prior clinical research or who had attended a respiratory clinic in NHS Tayside for severe asthma. This dataset includes all patients who have contacted the SCRR in order to help with participation in clinical trials, and who provided their consent for their contact details to be kept securely. Caldicott approval was obtained from the NHS Tayside Information Governance Team prior to any data collection for each individual study.

Airway measurements

Peak expiratory flow

Patients were asked to perform domiciliary early morning peak expiratory flow readings using a Mini-Wright peak flow meter (Clement Clarke, Harlow, UK) in the BISA study. Instructions comprised inhaling fully to total lung capacity followed by a forced exhalation through their mouth via the peak flow meter mouthpiece, with the best-of-three attempts recorded each day.

Spirometry

Spirometry was performed in all studies following the recommended guidelines from the European Respiratory Society.[170] Patients were instructed to inhale fully to total lung capacity, and then forcibly exhale all the way out to residual volume through the mouthpiece and filter attached to a mass-flow sensor. Measures of FEV_1, FVC and FEF_{25-75} could then be determined in absolute terms as well as compared to the normal population distribution as percentage of predicted (according to age, sex, height and ethnicity). All measurements were performed in triplicate to within a 5% tolerance, with the highest achieved value recorded. Studies used the MicroLab 3500 (Micro Medical; Chatham, UK) spirometer. Withholding times for asthmatic therapies prior to screening spirometry were as follows: antihistamines, 5 days; theophyllines, 2 days; LTRAs, 2 days; LABAs, 1 days; salbutamol or bricanyl, 6 hours.

Oscillometry

Impulse oscillometry (IOS) and airway oscillometry (AOS) measurements were performed according to published guidelines[171] and manufacturer's instructions. Oscillometry is an effort independent test of airway resistance and reactance performed during tidal breathing against a variety of sound or air waves of pre-specified amplitudes. Participants were required to hold both cheeks gently to prevent puffing and therefore shunting of the pressure wave. The impulses were applied over 20-30 seconds of tidal breathing. All measures were performed in triplicate with mean values calculated. The Masterscreen Impulse Oscillometer (Jaeger,

Höchberg, Germany) and TremoFlo (Thorasys, Montreal, Canada) were used where included as a study outcome measure. Accuracy of resistance measurements was confirmed on each day with a 3L calibration syringe (Masterscreen) or a standard 0.2 kPa/L/s resistance mesh (Tremoflo).

Mannitol challenge tests

Indirect bronchial challenge agents utilise and invoke the underlying inflammatory cascade in asthmatic airways to generate bronchoconstriction. We used mannitol bronchial challenge in the BISA trial. Mannitol is an osmotic agent that dries out the airway epithelium leading to release of inflammatory mediators and subsequent airway narrowing. Mannitol dry powder (Aridol, Pharmaxis, Sydney, Australia) bronchial challenge was performed as previously recommended.[172] Using the supplied dry powder inhaler device, patients serially inhaled doubling doses thus: 0, 5, 10, 20, 40, 80, 160, 160 and 160mg of mannitol. FEV_1 was measured 60 seconds after each inhalation, with the highest value of two recorded. The test ended once a 10% fall in FEV_1 was achieved, or when the maximum dose of 635mg had been given. The mannitol provocative dose to cause a 10% fall in FEV_1 (PD10) could then be calculated again using log-linear interpolation of the dose response curve.

Withholding times for asthmatic therapies prior to mannitol challenges were as follows: antihistamines, 2 days; theophyllines, 2 days; LTRAs, 2 days; LABAs, 1 days; salbutamol or bricanyl, 6 hours. Patients were given nebulised salbutamol 2.5mg immediately post-challenge to aid recovery.

Quality of life, asthma control and nasal symptom measures

Diary cards

In the BISA trial, patients were asked to complete diary cards, recording their best-of-three early morning measurement of peak expiratory flow rate (described above), rescue beta-2 agonist use and to assess their symptom burden. Symptoms were based on a rating scale of 0-3, comprising: 0 – no symptoms, 1 – mild symptoms, 2 – moderate symptoms, and 3 –

severe symptoms. These recordings were made during all run-in, treatment and washout periods.

Asthma quality of life questionnaire

The Juniper Asthma Quality of Life Questionnaire (AQLQ)[173] provides an overall score (comprising 32 questions and 4 domains), as well as the ability to tease out responses to individual domain components pertaining to: symptoms; activities; emotions; and environment. We used a smaller version called the mini-AQLQ (comprising 15 questions with the same 4 domains) in the BISA trial. Each question is on a 7-point scale; the responses are then averaged for each of the four domains, which themselves are subsequently averaged to provide the final score. A score of 7 indicates no impairment, and anything <7 indicates increasing impairment. Importantly, it has been shown to have a reliable minimal clinically important difference of 0.5.[174]

Asthma Control Questionnaire

The Asthma Control Questionnaire (ACQ)[175] comprises patient recall of the previous 7 days for: breathlessness; nocturnal wakening; symptoms on waking; activity limitation; wheeze; frequency of rescue beta-2 agonist use; and pre-bronchodilator FEV1 as %predicted. There is a 7-point scale for each domain with higher scores indicating worsening control. Once again, it has a minimal clinically important difference of 0.5, and shorter versions are available with similar clinical utility to the full version, which are simpler to administer, for example, in primary care.[65] Useful cut-off points have been established for the ACQ, where a score of ≤0.75 equates to 'well-controlled' asthma, and a score ≥1.5 determines 'not well-controlled' asthma.[176]

Total nasal symptom score

The total nasal symptom score (TNSS) is a brief questionnaire where patients rate individual nasal symptoms including rhinorrhoea, nasal congestion, nasal itching and sneezing using a 4-point categorical scale: 0, none; 1, mild; 2, moderate; and 3, severe. The overall score is obtained by summing the individual components with a maximum possible score of 12.[177]

Hyposmia and global visual analogue scales

The hyposmia visual analogue scale (VAS) and nasal global symptom VAS are simple tools used to determine a participant's subjective nasal symptom burden with higher scores suggesting more severe impairment. Participants are asked to draw a vertical line intersecting a standard horizontal 100-millimetre line.

Peak nasal inspiratory flow

In-Check peak nasal inspiratory flow (PNIF) meter (Clement Clarke International Ltd, Harlow, UK) was used to measure nasal airway obstruction noting the best-of-three value.[178]

Fractional exhaled nitric oxide

FeNO was measured in the BISA study using the portable NIOX VERO analyser (Circassia, Oxford, UK) and according to the American Thoracic Society and European Respiratory Society recommendations.[179] All measurements were taken prior to peak expiratory flow, spirometry and mannitol challenges as these may alter the FeNO values. A sustained exhaled breath lasting at least 8 seconds was required with a flow rate of 50 ml/s as guided by automatic feedback from the device.

Blood biomarkers

Peripheral blood measurements

Whole blood was obtained using aseptic technique by venepuncture from participants prior to mannitol challenge. Counts inclusive of blood eosinophil levels were measured using an ADVIA 2120s haematology system (Siemens Healthcare, Surrey, UK). Blood testing was also performed to detect presence of circulating levels of specific IgE antibodies to defined common aeroallergens [Fluorescence enzyme linked immunoassay (Phadia Immunocap 250)] including cat and dog dander, grass pollen, house dust mite and silver birch. All blood samples were processed and analysed in the haematology and immunology departments of Ninewells Hospital and Medical School, Dundee.

Serum eosinophil derived neurotoxin

Serum eosinophil derived neurotoxin (EDN) levels were measured by commercially available ELISA kits (MBL Medical and Biological Laboratories 7630, Nagoya, Japan) for human EDN. All samples were systematically diluted by 1:5 when needed and assayed following manufacturer instructions. The assay range after dilution was 3.0–200 ng/mL and the minimum detection limit was 0.62 ng/ml. Samples with an intra-assay coefficient of variation ≥15% were excluded from the analysis.

Imaging

HRCT scans were performed in volumetric mode with maximal inspiration, as per standard department protocol (128 slice CT Revolution EVO, GE Healthcare). CT reconstruction was performed in the lung window with a slice thickness of 1 - 1.25mm and no interval gap. Images were analysed in axial plane, with coronal and sagittal reconstruction used if necessary.

Mucus plugging

Both lungs were assigned 10 segments each as follows: right upper lobe (3 segments); right middle lobe (2 segments); right lower lobe (5 segments); left upper lobe (3 segments); lingula (2 segments); left lower lobe (5 segments). Mucus plugging was considered positive in this study if it completely occluded the lumen of any order bronchus, and it was out with 2cm of the pleural surface. A mucus plug score (MPS) was subsequently calculated with 0 denoting no MP and a maximum score of 20 to signify all areas contained at least 1 MP.[180]

Bronchial wall thickness

Airway lumen and total airway area were measured independently by two senior thoracic radiologists at four different bronchopulmonary segments: right apical; right lower lobe posterior basal; left apico-posterior and left lower lobe posterior basal. Wall area percentage (WA%) measurements were subsequently calculated from these values. The images were analysed in multiplanar reconstruction and measurements done in a plane perpendicular to

the corresponding segmental bronchi. Using free hand tool technique, the cross-sectional areas of airway lumen and total airway including wall were measured in mm^2.

Quality control

Sensitivity, specificity and coefficients of variation were monitored for individual batches of assays within the departmental laboratory. Measurements carried out within the main Ninewells Hospital haematology and immunology laboratories were subject to NHS quality standards.

Statistical methods

Data were analysed throughout the studies using several iterations of Statistical Products and Service Solutions (SPSS) statistical software for Windows, the most recent of these is version 27 (SPSS Inc., USA). A power calculation was derived within the BISA study. For all studies, statistical significance for any comparison was deemed to have been achieved below an alpha-error of 5% (two-tailed), with 95% confidence intervals for the mean change given when appropriate. Graphical representation of data was produced using GraphPad Prism version 6 (GraphPad Software Inc., USA). Further statistical detail is provided within each study.

Illustrations

Figures were created using Adobe Illustrator Artwork 26.0 for Windows.

Chapter 3: Small Airways Dysfunction and Biologics in Severe Asthma

Impact of biologic therapy on the small airways asthma phenotype

ABSTRACT

The small airways dysfunction (SAD) asthma phenotype is characterised by narrowing of airways <2mm in diameter between generations 8 and 23 of the bronchial tree. Recently, this has become particularly relevant as measurements of small airways using airway oscillometry for example, are strong determinants of asthma control and exacerbations in moderate to severe asthma. The small airways can be assessed using spirometry as forced expiratory flow rate between 25 and 75% of forced vital capacity (FEF_{25-75}) and has been deemed more accurate in detecting small airways dysfunction than forced expiratory volume in 1 second (FEV_1). Oscillometry as the heterogeneity in resistance between 5 and 20Hz (R5-R20), low frequency reactance at 5Hz (X5) or area under the reactance curve (AX) between 5Hz and the resonant frequency can also be used to assess the small airways. The small airways can also be assessed using the multiple breath nitrogen washout (MBNW) test giving rise to values including functional residual capacity, lung clearance index and ventilation distribution heterogeneity in the conducting (Scond) and the acinar (Sacin) airways. The ATLANTIS group showed that the prevalence of small airways disease in asthma defined on FEF_{25-75}, oscillometry and MBNW all increased with progressive GINA asthma disease stages. As opposed to topical inhaler therapy that might not adequately penetrate the small airways, it is perhaps more intuitive that systemic anti-inflammatory therapy with biologics targeting downstream cytokines and upstream epithelial anti-alarmins may offer a promising solution to SAD. Here we therefore aim to appraise the available evidence for the effect of anti-IgE, anti-IL5(Rα), anti-IL4Rα, anti-TSLP and anti-IL33 biologics on small airways disease in patients with severe asthma.

INTRODUCTION

The small airways dysfunction (SAD) asthma phenotype is characterised by narrowing of airways <2mm in diameter between generations 8 and 23 of the bronchial tree.[181] Recently, this has become particularly relevant as measurements of small airways using airway oscillometry for example, are strong determinants of asthma control and exacerbations in

moderate to severe asthma.[182] The small airways can be assessed using spirometry as forced expiratory flow rate between 25 and 75% of forced vital capacity (FEF_{25-75}) and has been deemed more accurate in detecting small airways dysfunction than forced expiratory volume in 1 second (FEV_1).[183] Having said that FEF_{25-75} is rather volume dependent in terms of ensuring patients breathe out all the way to residual volume and as such is considered to be more variable.

Oscillometry as the heterogeneity in resistance between 5 and 20Hz (R5-R20), low frequency reactance at 5Hz (X5) or area under the reactance curve (AX) between 5Hz and the resonant frequency can also be used to assess the small airways.[184] X5 and AX are thought to reflect peripheral lung compliance which is reduced in patients with SAD. Oscillometry has advantages over spirometry in being effort independent, being more associated with type 2 inflammation and having higher sensitivity with regards to bronchodilator responses.[7,185] In patients with moderate to severe persistent asthma not taking biologics the presence of abnormal values of either R5-R20 ≥0.10kPa/L/s or AX ≥1.0kPa/L were associated with worse disease control as ACQ score.[186] Using computational modelling, it has previously been shown that R5-R20 is a direct measure of anatomical narrowing of the small airways.[158] Contemporaneously, it has been determined that combining both spirometry and oscillometry measurements might better identify moderate to severe asthma patients with worse control and more frequent exacerbations.[187,188]

The small airways can also be assessed using the multiple breath nitrogen washout (MBNW) test giving rise to values including functional residual capacity, lung clearance index and ventilation distribution heterogeneity in the conducting (Scond) and the acinar (Sacin) airways.[189] The ATLANTIS group showed that the prevalence of small airways disease in asthma defined on FEF_{25-75}, oscillometry and MBNW all increased with progressive GINA asthma disease stages.[155]

The peripheral airways have previously been termed the quiet zone of the lung because they are difficult to assess and treat. Conventional high doses of inhaled corticosteroids have been shown to be relatively ineffective in managing distal lung inflammation measured by alveolar nitric oxide.[190] This is likely attributed to aerosols comprising a larger particle size that have a predilection to deposit in the large airways.[191] In one study, adding extra-fine HFA-BDP on top of high dose conventional particle fluticasone/salmeterol conferred no improvement in

oscillometry small airways function or alveolar NO in patients with severe persistent asthma.[192] Over the past decade, type 2 biologic therapies have been shown to significantly improve exacerbations and other clinical outcomes such as disease control, pulmonary function and type 2 biomarkers.[1,2]

Here we therefore aim to appraise the available evidence for the effect of systemic biologic therapies on small airways disease in patients with severe asthma. We searched PubMed and Google Scholar for terms including "small airways", "omalizumab", "mepolizumab", "benralizumab", "reslizumab", "dupilumab", "tezepelumab", "itepekimab", "FEF_{25-75}", "oscillometry" and "multiple breath nitrogen washout" with abstracts and case reports excluded. The aim here is not to perform a systematic review or meta-analysis as the investigated outcomes in these cited studies are too heterogenous to amalgamate. The essential premise here is that the systemic route of administration would facilitate delivery of biologics to the whole lung including the peripheral airways in the same way as oral corticosteroids in patients who are refractory to high dose ICS. Given that the airway mucosal surface area is proportionately much greater in the distal compared to proximal lung, systemic delivery of biologics appears to be a cogent way for treating all of the type 2 inflammation in asthmatic airways. Indeed, this may be one of the reasons why systemic biologics are so successful at improving control in severe asthma patients despite the use of high dose inhaled combination therapy.

OMALIZUMAB

Omalizumab is a recombinant humanised anti-IgE monoclonal IgG1 antibody that blocks the binding of free IgE to its high affinity FcεRI receptor on mast cells and basophils.[193] It has the secondary action of binding to membrane bound IgE (mIgE) on mIgE-expressing B cells resulting in downregulation of IgE production.[194] A Cochrane review has demonstrated significant reductions in exacerbations and hospitalisations in moderate to severe asthma.[71] As FcεRI expression is increased throughout the large and small airways in severe asthma,[195] one might postulate that a systemic therapy such as omalizumab would confer additional benefit to allergic patients only taking topical inhaler therapy.

A retrospective cohort study (n=110) in adult patients with severe eosinophilic allergic asthma showed that omalizumab significantly improved FEF_{25-75} by 8.3% over 52 weeks.[196] Another real life retrospective clinical study (n=20) of severe asthma patients demonstrated that omalizumab significantly improves FEF_{25-75}% by 6% but not FEV_1% by 4%, over 44 weeks along with clinically significant reductions in exacerbations and ACQ scores.[159] A prospective observational study (n=26) also highlighted an improvement in alveolar nitric oxide levels in severe asthmatics following 48 weeks of omalizumab indicating a potential therapeutic effect on small airways type 2 inflammation.[197] This is important as uncontrolled small airways inflammation is related to airway remodelling and progression of disease.[198] Additionally, patients with aspirin exacerbated respiratory disease (AERD) generally have higher levels of type 2 inflammation,[3] and in one small case series (n=4) such patients also experienced improvements in FEF_{25-75} by 30%.[199] No studies have been performed looking at the effect of omalizumab therapy on other measures of small airways disease.

MEPOLIZUMAB, RESLIZUMAB AND BENRALIZUMAB

Mepolizumab and Reslizumab are humanised IgG1κ and IgG4κ monoclonal antibodies respectively that exert its effect by inhibiting interleukin 5 attachment to the IL5Rα receptor on eosinophils.[200,201] Benralizumab is a humanised IgG1κ monoclonal antibody that binds to the IL5Rα receptor on eosinophils to prevent IL5 activation.[202] Through this shared mechanism of action, suppression or depletion of eosinophilic activation, proliferation and migration is achieved. Due to higher expression of IL5 mRNA in the small airways (<2mm diameter) in asthmatics, one might expect mepolizumab, reslizumab and benralizumab therapy to be effective in SAD.[203]

The phase 3b RCT MUSCA demonstrated significant improvements in FEF_{25-75} amounting to 0.123L/s after 24 weeks of mepolizumab vs placebo in n=551 patients with severe eosinophilic asthma.[204] Although this is the largest study investigating the effect of mepolizumab in small airways, MUSCA was not powered a priori on FEF_{25-75}. To support this, two retrospective studies (n=134 and n=105) independently demonstrated a significant improvement in FEF_{25-75}% with mepolizumab in severe eosinophilic asthma patients with respective improvements of 9.8% and 8.1%.[205,206] Smaller observational studies[159,207] (n=31 and n=30) have shown no improvement in FEF_{25-75}% after 24 to 44 weeks of mepolizumab. However, the mean baseline

FEF_{25-75}% in these smaller studies were higher and therefore there may have been less room for improvement.

In a prospective study (n=18), it was shown that oscillometry low frequency reactance as X5, a measure of peripheral lung compliance, significantly improved by 74% one month post mepolizumab therapy in severe eosinophilic asthma.[208] However, another retrospective study in severe asthmatics (n=30) showed no improvements in R5-R20 or AX following 10 months of mepolizumab.[159] These studies are likely to be underpowered to draw any meaningful conclusions. One prospective cohort study (n=20) showed a significant improvement in small airway function after 26 weeks with mepolizumab measured by ventilation heterogeneity as Sacin using MBNW in patients with severe eosinophilic asthma.[209]

In a phase 3 randomised controlled trial (RCT) (n=205),[210] there was a borderline significant trend for iv reslizumab 3mg/kg to improve FEF_{25-75} over 16 weeks by 0.233L/s vs placebo, exceeding the established biological variability in severe asthma of 0.21L/s[5] to infer a clinically relevant treatment effect. An open label extension study (n=1051) has shown that these FEF_{25-75}% improvements persist up to 96 weeks on reslizumab in patients with moderate-to-severe eosinophilic asthma.[211] Post hoc analysis of two phase 3 RCTs (n=723) in severe eosinophilic asthma showed that reslizumab significantly improves FEF_{25-75} over placebo with a mean difference 0.128L/s.[212] Although reslizumab is used in clinical practice to a lesser extent, we postulate that these encouraging results can possibly be extrapolated to mepolizumab due to the shared immunological pathway. No studies to date have been performed on reslizumab looking at oscillometry or MBNW outcomes.

A multicentre retrospective observational study[213] (n=137) looking at patients with severe eosinophilic asthma demonstrated significant improvements in FEF_{25-75}% amounting to 17% after 24 weeks of benralizumab. Another real-life retrospective observational study[160] (n=22) showed that benralizumab improved FEF_{25-75} by 0.82L/s over 24 weeks in severe allergic eosinophilic asthma patients greatly exceeding the biological variability value[5] for a clinically relevant effect. In one prospective observational study with benralizumab in severe asthma[214] (n=19) no improvements in R5-R20, X5 and AX were observed after 24 weeks. Pointedly, patients in this study started with normal small airways function and therefore one would perhaps not expect any improvement.

DUPILUMAB

Dupilumab is a humanised IgG4 monoclonal antibody that targets the IL4Rα receptor to mediate IL4 and IL13 activity.[215] Interestingly, IL4 and IL13 but not IL5 have been shown to induce hyperresponsiveness in isolated small airways.[138] Additionally, more IL4 mRNA expression has been found in the small airways of asthmatic versus non-asthma patients.[203]

The phase 3 LIBERTY ASTHMA QUEST trial[216] (n=1902) in uncontrolled moderate-to-severe asthma showed that FEF_{25-75} significantly improved by 0.16L/s following 52 weeks of dupilumab treatment compared to placebo. In this regard, a phase 2 RCT[145] (n=148) in moderate-to-severe asthma also showed that dupilumab improved FEF_{25-75} by 0.19L/s compared to placebo over 12 weeks albeit the significance was not reported here since it was not the primary outcome. In another prospective cohort study[217] (n=20) of severe asthma patients with nasal polyps treated with dupilumab for 4 weeks there was a significant improvement in FEF_{25-75} of 0.33L/s exceeding biological variability. In terms of airway oscillometry, one retrospective study[218] (n=62) in mild-to-moderate asthma with concomitant CRSwNP showed that 3 months of dupilumab therapy did not significantly change X5.

TEZEPELUMAB

Tezepelumab is a humanised IgG2λ monoclonal antibody that blocks the upstream epithelial alarmin thymic stromal lymphopoietin (TSLP) from interacting with the TSLP receptor complex resulting in dampening of the type 2 inflammatory response.[219] The phase 2 CASCADE trial[129] (n=110) in moderate-to-severe uncontrolled asthma demonstrated no improvement in FEF_{25-75} or R5-R20 over placebo although interestingly tezepelumab resulted in a 0.56kPa/L improvement in AX that exceeds the biological variability value of 0.39kPa/L in severe asthma.[5]

ITEPEKIMAB

Itepekimab is a humanised IgG4 monoclonal antibody with anti-alarmin activity against IL-33 resulting in suppression of type 2 inflammation.[145] In a phase 2 RCT of moderate-to-severe asthmatics (n=148),[145] itepekimab was shown to improve FEF_{25-75} by 0.170L/s over 12 weeks

compared to placebo, which did not exceed the biological variability value. In this regard, the same phase 2 RCT[145] with combined itepekimab and dupilumab conferred a 0.120L/s improvement in FEF_{25-75} over placebo which was numerically less than for itepekimab or dupilumab monotherapy alone. This suggests that merely blocking more type 2 inflammatory pathways may not be the answer. The effect of various biologic therapies on FEF_{25-75} is summarised in tabular form (table 4).

Table 4 *Summary of current evidence base for the effect of biologics on forced expiratory flow rate between 25 and 75% of forced vital capacity (FEF_{25-75})*

Study	Type of study	Biologic	Numbers of patients	Baseline FEF_{25-75} (L/s or %)	Duration of therapy	Absolute Improvement (L/s or %) *	P value
Huang et al[185]	Retrospective	OMA	n=110	55.1%	52 wks	8.3%	<0.001
Chan et al[148]	Retrospective	OMA	n=20	43%	44 wks	6%	<0.05
Chupp et al[193]	RCT	MEPO	n=551	0.894L/s	24 wks	0.123L/s	0.002
Sposato et al[194]	Retrospective	MEPO	n=134	37.4%	47 wks	9.8%	<0.001
Maglio et al[195]	Retrospective	MEPO	n=105	32.7%	24 wks	8.1%	<0.001
Yılmaz et al[196]	Retrospective	MEPO	n=31	45.1%	24 wks	3.6%	NS
Chan et al[148]	Retrospective	MEPO	n=30	46%	44 wks	6%	NS
Bjermer et al[199]	RCT	RESLI	n=205	N/A	16 wks	0.233L/s	0.055
Murphy et al[200]	Open label extension	RESLI	n=1051	1.6 L/s	96 wks	0.4L/s	N/A
Virchow et al[201]	Post hoc of 2 RCTs	RESLI	n=723	1.55L/s	52 wks	0.128L/s	0.005
Nolasco et al[202]	Retrospective	BENRA	n=137	38%	24 wks	17%	<0.001
Pelaia et al[149]	Retrospective	BENRA	n=22	0.6L/s	24 wks	0.82L/s	<0.001
Castro et al[205]	RCT	DUPI	n=1902	1.113L/s	52 wks	0.145L/s	<0.001
Wechsler et al[133]	RCT	DUPI	n=148	N/A	12 wks	0.19L/s	<0.05
Pelaia et al[206]	Prospective	DUPI	n=20	1.47L/s	4 wks	0.33L/s	<0.01
Diver et al[119]	RCT	TEZE	n=110	1.26L/s	28 wks	-0.01L/s	NS
Wechsler et al[133]	RCT	ITEPE	n=148	N/A	12 wks	0.17L/s	NS
Wechsler et al[133]	RCT	ITEPE + DUPI	n=148	N/A	12 wks	0.12L/s	NS

**Absolute values provided as improvements compared to placebo for RCTs*

BENRA = benralizumab; DUPI = dupilumab; ITEPE = itepekimab; MEPO = mepolizumab; N/A = not available; NS = non-significant; OMA = omalizumab; RCT = randomised controlled trial; RESLI = reslizumab; TEZE = tezepelumab

CONCLUSIONS

Prospective RCTs with various biologics are now indicated which are properly powered on small airway outcomes, where patients are selected a priori on the basis of having clinically relevant degrees of SAD. We would duly suggest that such patients might exhibit values for spirometry as FEF_{25-75} <50%, or oscillometry as X5 <-0.20kPa/L/s, R5-20 ≥0.10kPa/L/s or AX ≥1.0kPa/L given that such values are associated with poor control and more frequent exacerbations.[187,220] Ideally, future studies should take into consideration z-scores for FEF_{25-75} to account for differences in age and height although in a real life busy clinic it is perhaps more pragmatic to use absolute cut offs. Oscillometry in particular is easy to perform and effort dependent with validated biological variability values and is therefore eminently suitable for powering such studies in the first instance. In this regard, the ongoing SASAM trial (NCT05040997) is using small airways disease measured by spirometry, body plethysmography, single and multiple breath nitrogen washout and impulse oscillometry as novel endpoints and distinct targets for mepolizumab. The problem for such a trial is deciding on which of the SAD outcomes should be selected as the primary end point in that patients with asthma may for example have relatively well-preserved spirometry with abnormal oscillometry.[187,220] Another study (NCT03976310) is currently looking at the effects of benralizumab in air trapping, which can be considered a surrogate for small airways disease,[188] on high resolution computed tomography imaging as the primary outcome. Tezepelumab is also presently being studied (NCT05280418) to look at its effect in ventilation heterogeneity on hyperpolarised ^{129}Xe magnetic resonance imaging as the primary outcome.

Oscillometry bronchodilator response in adult moderate to severe eosinophilic asthma patients

AIM: TO COMPARE BRONCHODILATOR RESPONSES FOR AIRWAVE OSCILLOMETRY AND SPIROMETRY IN SEVERE ASTHMA

The presence of bronchodilator response (BDR) is one of the key hallmarks in diagnosing asthma and is traditionally defined as a >200ml and >12% improvement in spirometry forced expiratory volume in 1 second (FEV_1) following short acting beta agonist therapy. Patients who demonstrate BDR typically have higher levels of airway inflammation, poorer asthma control and a greater spirometric response to inhaled corticosteroid (ICS) therapy.[221-223]

Airway oscillometry is an effort-independent tidal breathing manoeuvre that also assesses small airway function through measuring differences in resistance between 5 and 20 Hz (R5-R20), reactance at 5 Hz (X5) and area under reactance curve (AX).[159] It has previously been demonstrated that oscillometry BDR is related to asthma control,[224] and that R5-R20 and AX bronchodilator response display greater sensitivity compared to that of FEV_1 or FEF_{25-75} in response to salbutamol in mild to moderate asthma patients.[225] In this prospective cohort study, we aim to elucidate similarities and differences in BDR for spirometry and oscillometry in patients with poorly controlled severe asthma with type 2 inflammation.

33 severe asthma patients attending the Scottish Centre for Respiratory Research for screening into a separate clinical trial (EudraCT No. 2019-003763-22) were enrolled into this study between December 2020 and October 2021. Prior to their appointment, all patients were instructed to withhold their SABA for 6 hours; ICS for 12 or 24 hours depending on dosing frequency, long-acting beta-agonists (LABA) for 12 or 24 hours; long-acting muscarinic antagonists (LAMA) for 12 or 24 hours; theophylline for 48 hours; leukotriene receptor antagonists (LTRA) for 48 hours and antihistamines for 5 days. No patients were taking biologics at enrolment. Fractional exhaled nitric oxide (FeNO) was measured using NIOX VERO (Circassia, Oxford, UK) according to the manufacturer's instructions and ATS/ERS guidelines. Spirometry (Micromedical, Chatham, UK) was performed according to ERS guidelines. Thorasys TremoFlo Airwave Oscillometry system measurements were performed in triplicate to assess oscillometry according to the ERS guidelines with oscillometry always performed prior to spirometry. Blood testing was performed to detect levels of peripheral blood

eosinophils (PBE) and circulating levels of specific IgE antibodies [Fluorescence enzyme linked immunoassay (Phadia Immunocap 250)] to defined common allergens including house dust mite, grass, cat, dog and silver birch. Asthma control was determined using the 6-point asthma control questionnaire (ACQ) and mini asthma quality of life questionnaire (mini-AQLQ). All patients were subsequently administered 400µg salbutamol via a pMDI through an aerochamber spacer device (Trudell Medical UK Ltd) with oscillometry and spirometry measurements repeated after 15 minutes. Statistical analysis was performed using SPSS version 27 and graphs were prepared with GraphPad Prism 6 (GraphPad Software Inc). Data were assessed for normality with Boxplots prior to analysis. Paired Student's T tests with a two tailed alpha error set at 0.05 were implemented to evaluate any significant differences in pulmonary function pre- and post-salbutamol. Independent Student's T tests were also used to compare pre-bronchodilator spirometry, oscillometry, type 2 biomarkers and ACQ in those patients with or without spirometry, oscillometry or combined-critieria defined BDR. Pearson's correlation coefficients were computed to assess the relationship between percentage differences for spirometry and oscillometry. Data were all normally distributed. The standardised response mean (SRM) expresses the signal to noise ratio as mean change divided by SD (SRM≥0.80 are considered highly sensitive). Ethical approval was obtained through the East of Scotland research ethics service.

The mean baseline demographic data are shown in table 5. One patient was taking a daily oral prednisolone dose of 1mg.

Table 5 Baseline patient demographics

Gender (F/M)	18/15	**Ex-smoker (%)**	39
Age (yrs)	52 (3)	**FEV_1%**	76 (4)
BMI (kg/m^2)	31 (1)	**FEF_{25-75}%**	39 (4)
LABA (%)	79	**FVC%**	98 (3)
LAMA (%)	52	**FEV_1/FVC**	0.63 (0.02)
LTRA (%)	64	**R5 (kPa/L/s)**	0.59 (0.04)
THEO (%)	21	**R20 (kPa/L/s)**	0.40 (0.02)
OAH (%)	70	**R5-R20 (kPa/L/s)**	0.19 (0.03)
CROMO (%)	3	**X5 (kPa/L/s)**	-0.33 (0.05)
INAH (%)	12	**AX (kPa/L)**	3.77 (0.64)
INS (%)	48	**Fres (Hz)**	24.00 (1.42)
ACQ	3.0 (0.1)	**ICS dose (µg)**	1875 (54)
Mini-AQLQ	3.2 (0.2)	**FeNO (ppb)**	54 (8)
		Total IgE (kU/L)	388 (43)
		PBE (cells/µl)	505 (62)

Mean (SEM)

When comparing pre- and post-bronchodilator measurements (Table 6), spirometry and oscillometry values were all statistically significant ($p<0.001$). Similar outcomes resulted from repeating the analysis for those patients with AHR to mannitol (n=21). The greatest improvements after bronchodilation (expressed as % of baseline) were observed for R5-R20 (37.9%) and AX (53.5%) whilst the lowest improvements were demonstrated for FVC (4.1%) and FEV_1 (10.4%). SRMs for FEV_1, R5, X5, AX and F_{res} were all highly sensitive (>0.8) although was highest for FEV_1 (Table 6). Improvements in FEF_{25-75}% and R5-R20% were moderately correlated ($r=0.47$; $p=0.006$).

Table 6 *Mean absolute and percentage differences and standardised response means for pre- and post-bronchodilator oscillometry and spirometry measurements*

	Mean difference (95%CI)	**% difference (95%CI)**	**P value**	**SRM**
FEV_1 (L)	0.231 (0.168 – 0.295)	10.4 (7.5 – 13.2)	<0.001	1.29
FEF_{25-75} (L/s)	0.356 (0.190 – 0.523)	25.9 (13.8 – 38.0)	<0.001	0.76
FVC (L)	0.142 (0.066 – 0.219)	4.1 (1.9 – 6.2)	<0.001	0.66
R5 (kPa/L/s)	0.12 (0.08 – 0.16)	20.1 (13.5 – 26.8)	<0.001	1.07
R20 (kPa/L/s)	0.05 (0.02 – 0.07)	11.5 (5.8 – 17.1)	<0.001	0.73
R5-R20 (kPa/L/s)	0.07 (0.05 – 0.10)	37.9 (24.4 – 51.5)	<0.001	0.99
AX (kPa/L)	2.02 (1.16 – 2.87)	53.5 (30.8 – 76.2)	<0.001	0.84
X5 (kPa/L/s)	0.11 (0.07 – 0.16)	33.7 (20.0 – 47.4)	<0.001	
F_{res} (Hz)	4.60 (2.55 – 6.65)	19.5 (10.8 – 28.1)	<0.001	0.90

CI = confidence interval; SRM = standardised response means

In our cohort of severe asthma patients 11/33 (33%) had a positive BDR when using the standard FEV_1 criteria of >200ml and >12% improvement post-salbutamol. When using recently recommended oscillometry BDR criteria namely R5≥29% or X5≥45%,[226] 12/33 (36%) had a positive BDR (table 6). No significant differences in spirometry, oscillometry, asthma control or type 2 biomarkers were noted when using spirometry or oscillometry BDR criteria separately.

Table 7 *Comparisons of spirometry, oscillometry, asthma control and type 2 biomarkers according to presence or absence of bronchodilator response using FEV_1 or oscillometry criteria*

	FEV_1 BDR (n=11) vs non-BDR (n=22)	**Oscillometry BDR (n=12) vs non-BDR (n=21)**
FEV_1 (L)	2.24 vs 2.23	2.40 vs 2.14
FEF_{25-75} (L/s)	1.14 vs 1.49	1.46 vs 1.33
FVC (L)	3.85 vs 3.34	3.82 vs 3.33
R5 (kPa/L/s)	0.70 vs 0.53	0.67 vs 0.54
R20 (kPa/L/s)	0.45 vs 0.38	0.42 vs 0.40
R5-R20 (kPa/L/s)	0.25 vs 0.16	0.25 vs 0.15
AX (kPa/L)	5.03 vs 3.14	5.02 vs 3.05
X5 (kPa/L/s)	-0.37 vs -0.32	-0.37 vs -0.32
F_{res} (Hz)	26.16 vs 23.04	27.47 vs 22.16
ACQ	3.3 vs 2.9	2.9 vs 3.1
Mini AQLQ	3.1 vs 3.2	3.4 vs 3.1
FeNO (ppb)	74 vs 40	45 vs 55
PBE (cells/µl)	474 vs 522	**338 vs 598***

*BDR = bronchodilator response; *p<0.05*

To our knowledge, this is the first study comparing BDR for oscillometry and spirometry in patients with poorly controlled severe asthma with type 2 inflammation. Respiratory impedance values for BDR in healthy volunteers have previously been documented, but in contrast, our cohort of patients had evidence of severe asthma. Notably, the mean baseline FEV_1 improved by 231ml and 10.4% pre- versus post-salbutamol. One possible explanation for the lack of spirometry BDR in this study perhaps could be related to the fact that severe asthma is more associated with airway remodeling and fixed airflow obstruction than mild-to-moderate asthma.[227]

One recent retrospective study observed that oscillometry BDR was associated with poor asthma control and was more sensitive than spirometry BDR.[228] However, this study did not investigate small airways resistance using R5-R20 or FEF_{25-75}. In the present study, we have prospectively demonstrated that both reactance (X5 and AX) and resistance measurements (R5-R20) in addition to FEF_{25-75} showed the greatest improvements in BDR compared to FEV_1 (Table 6).

Improvements in FEF_{25-75}% and R5-R20% were moderately correlated. This is intuitive as both measurements are considered markers for SAD. Indeed, BDR values were highest for measurements of SAD including FEF_{25-75}, R5-R20, X5 and AX whilst FEV_1, FVC, R5, R20 and F_{res} had relatively lower BDR (Table 6). The findings from this study are clinically relevant as biologic therapy has previously been shown to improve FEF_{25-75} and R5-R20 in patients with severe asthma along with its well established effects on better asthma control.[159]

We appreciate our study is limited in terms of a relatively small sample size and results from a single Scottish centre and therefore larger multicentre studies are indicated to validate our results including patients taking biologics. However, this is the first prospective study to assess oscillometry BDR in severe asthma patients with type 2 inflammation and therefore we hope this novelty will lead to further studies in this rapidly evolving area.

In conclusion, measurements for small airways dysfunction including FEF_{25-75} and oscillometry demonstrated greater percentage improvements in bronchodilator response compared to baseline than FEV_1 and FVC in severe asthma patients. Standardised response means for FEV_1, R5, X5, AX and F_{res} were all highly sensitive although was highest for FEV_1.

Addendum

On reflection, I should have further explored the dose of salbutamol used to elicit the bronchodilator response (BDR) in this study. For instance, one prospective study[229] showed that FEV_1 continues to improve up to and including a cumulative dose of 3,200µg of salbutamol, even in patients previously taking long-acting beta agonist (LABA) therapy. However, one might argue that 400µg of salbutamol is more akin to the standard dose used in real life clinical practice[221] due to safety concerns with higher exposure. Additionally, the

mean FEV_1 in this study[229] was 57.6% which perhaps allowed for much more room improvement compared to 76% as in our study.

Another interesting aspect was the duration of time between pre- and post-bronchodilator pulmonary function measurements. In this regard, one study looking at asthma patients with a mean FEV_1 of 78% predicted[230] demonstrated continual improvement in FEV_1 up to and including 30 minutes following salbutamol 200µg. However, in this study, BDR was assessed following methacholine bronchial challenge and therefore one might hypothesise that recovery responses were somewhat exaggerated. Nevertheless, future assessments of oscillometry could look at the longitudinal effect of salbutamol on BDR to properly characterise this phenomenon.

Lastly, our LABA withholding time of 12 to 24 hours may not have been sufficient to determine maximal bronchodilation due to prolonged receptor occupancy and cross tolerance to salbutamol as shown in this study[229] where patients taking salmeterol had a blunted BDR to salbutamol after 12 hours of LABA withholding. However, our withholding times are in keeping with current practice[228] and it is unethical to deliberately withhold efficacious medication for a prolonged duration of time.

Repeatability of impulse oscillometry in patients with severe asthma

AIM: TO DETERMINE REPEATABILITY AND BIOLOGICAL VARIABILITY VALUES FOR IMPULSE OSCILLOMETRY IN SEVERE ASTHMA

Impulse oscillometry (IOS) involves an effort independent tidal breathing manoeuvre to determine the presence or absence of small airways dysfunction (SAD), defined as raised peripheral airway resistance (difference in resistance between 5 and 20Hz (R5-R20)) and/or raised peripheral airway reactance (area under the reactance curve (AX)).[171] IOS has clear advantages over spirometry especially in patients where accurate forced volumetric measurements may be difficult or impossible to achieve, and has proven its utility in asthma and COPD although work is still required to determine normal reference ranges and the minimal clinically important difference (MCID) for changes in measurements.[231]

In medical statistics the coefficient of variation (CV) is commonly used as a measure of precision and repeatability of data and additionally can be utilised to assess variability between two different devices that perform the same task irrespective of their units of measurement.[232] CV is calculated by dividing the sample standard deviation by the sample mean and is usually expressed as a percentage. A larger CV value reflects higher variability and therefore lower consistency between repeated measurements in a given subject. Biological variability (BV), a measurement of natural fluctuation, can be calculated as the one sided 97.5% CI. Its value can be used as a surrogate for the minimal change that must be exceeded for a clinically significant treatment effect or MCID to occur.

Therefore, we performed a retrospective study to compare the within-subject variability of IOS and spirometry measurements over two timepoints (T1 and T2) in 42 severe asthma patients attending our specialist NHS clinic who underwent no change in treatment over the period of assessment. Fractional exhaled nitric oxide (FeNO) was measured using NIOX VERO (Circassia, Oxford, UK) according to manufacturer's instructions and ATS/ERS guidelines.[179] Spirometry (Micromedical, Chatham, UK) was performed according to European Respiratory Society (ERS) guidelines.[170] IOS (Masterscreen, Carefusion Hoechberg, Germany) measurements were performed in triplicate according to the ERS guidelines with IOS always performed prior to spirometry.[171] Data were first analysed for normality using normality plots

and paired sample T tests were used to determine statistical significance with alpha error (two tailed) set at 0.05. Pearson's correlation coefficients were computed to assess the relationship between CVs for IOS and spirometry. Data were all normally distributed. Biological variability and coefficients of variation were calculated for each variable and the means (95% CI) presented in Table 9. The within subject absolute biological variability was calculated as a one sided 97.5%CI value. Other 95%CI were calculated as two-sided values. Caldicott Guardian approval was obtained prior to all data collection.

The mean (SEM) baseline demographic data are shown in table 8. Our patients had preserved FEV_1 (mean %pred) but evidence of SAD as evidenced by reduced FEF_{25-75} (%pred) but raised R5-R20 (kPa/L/s) and AX (kPa/L). Moreover, our severe asthma patients had a mean ACQ score of 2.1 and 4 asthma exacerbations requiring oral corticosteroids (OCS) in the past year denoting poor control despite a high beclomethasone diproprionate (BDP) equivalent ICS dose. The mean time in pulmonary function, ACQ score and FeNO measurements between T1 and T2 was 321 days (SD 208; Range 63 - 1085). PBE counts were averaged over the preceding 6 months whilst FeNO results were obtained on the same day as pulmonary function and ACQ.

Table 8 *Baseline patient demographics*

Gender (F/M)	27/15	**Ex-smoker (%)**	17
Age (yrs)	53 (2)	**Current smoker (%)**	7
BMI (kg/m^2)	32 (1)	**FEV_1%**	87 (4)
LABA (%)	95	**FEF_{25-75}%**	51 (4)
LAMA (%)	57	**FVC%**	106 (3)
LTRA (%)	52	**R5 (kPa/L/s)**	0.55 (0.03)
THEO (%)	36	**R20 (kPa/L/s)**	0.42 (0.02)
OAH (%)	60	**R5-R20 (kPa/L/s)**	0.14 (0.02)
INAH (%)	12	**AX (kPa/L)**	1.39 (0.21)
INS (%)	55	**Fres (Hz)**	17.61 (1.01)
Anti-IgE (%)	5	**ICS dose (µg)**	1850 (43)
Anti-IL5 (%)	12	**FeNO (ppb)**	26 (3)
ACQ	2.1 (0.3)	**PBE (cells/µl)**	404 (39)
		AERD (%)	14
		CRSwNP (%)	38

Mean (SEM)

No statistically significant differences were detected when comparing spirometry, IOS, ACQ, or PBE count. Table 9 depicts the mean absolute and percentage changes with two-sided 95%CI, CVs with two-sided 95%CI and BVs with one sided 97.5%CI in pulmonary function. For spirometry, FEV_1, FVC and FEF_{25-75} had CVs ranging between 6.9% to 20.3%, whilst for IOS, CV values for R5, R20, F_{res} and AX were between 12.9% to 39.2%. FEF_{25-75} and AX had the highest CV values amounting to 20.3% and 39.2%. Differences in ACQ scores exceeded 0.5 in 71% of patients between T1 and T2.

Table 9 *Mean absolute and percentage changes, coefficient of variation and biological variability in pulmonary function, ACQ and type 2 biomarkers between timepoints*

	Mean absolute change (95% CI)	Mean percentage change (95% CI)	Mean CV (95% CI)	Biological Variability (97.5% CI)	P value
FEV_1 (L)	0.100 (-0.048 – 0.250)	4% (-2 – 10.1)	10.1% (6.7 – 13.5)	0.15	0.179
FEF_{25-75} (L/s)	0.122 (-0.088 – 0.332)	6.9% (-5.2 - 19)	20.3% (14.1 – 26.5)	0.21	0.247
FVC (L)	0.118 (-0.026 – 0.261)	3.3% (-0.8 – 7.1)	6.9% (4.6 – 9.2)	0.15	0.106
R5 (kPa/L/s)	-0.01 (-0.07 – 0.06)	-1.8% (-12.7 – 10.9)	16.1% (11.6 – 20.6)	0.07	0.868
R5-R20 (kPa/L/s)	-0.02 (-0.06 – 0.02)	16.5% (-45.8 – 12.7)	33.1% (19.5 – 46.7)	0.04	0.241
R20 (kPa/L/s)	0.02 (-0.01 – 0.05)	4.8% (-2.4 – 11.9)	12.5% (9.2 – 15.8)	0.03	0.260
AX (kPa/L)	-0.17 (-0.55 – 0.22)	-12.2% (-39.6 – 15.8)	39.2% (28.9 – 49.6)	0.39	0.393
F_{res} (Hz)	-0.11 (-1.61 – 1.39)	-0.6% (-9.1 – 7.9)	14% (9.4 – 18.5)	1.5	0.880
ACQ	-0.1 (-0.7 – 0.5)	5.7% (-35 – 23.6)	46.7% (30 – 63.3)	0.6	0.688
PBE (cells/μL) *	-35 (-138 – 69)	-8.8% (-35.1 – 17.5)	37.7% (25.1 – 50.3)	104	0.500
FeNO (ppb)#	-17 (-32 – -2)	-66.8 (-125.6 – -7.9)	43.7% (33 – 54.4)	15	0.028

*AX = area under the reactance curve; CV = coefficient of variation; FeNO = fractional exhaled nitric oxide; FEF_{25-75} = forced mid expiratory flow rate between 25 and 75% of forced vital capacity (FVC); FEV_1 = forced expiratory volume in 1 second; F_{res} = resonance frequency; PBE = peripheral blood eosinophils; R5 = resistance at 5Hz; R20 = resistance at 20Hz. Within subject biological variability was calculated as a one-sided 97.5%CI. Other 95%CI were two-sided. *n=35 #n=25*

Weak correlations in variability were detected for FEF_{25-75} with AX (r=0.37; p=0.015) and Fres (r=0.35; p=0.025) but not for R5-R20 (r=0.12; p=0.464) between the two timepoints.

With regards to biological variability for AX, a one-sided 97.5%CI of 0.39 kPa/L infers that a change exceeding this is required to represent a clinically meaningful response. Notably, our CVs for FEV_1 (10.1%) and FEF_{25-75} (20.3%) were comparable to that of previous literature.[233] This perhaps suggests that one should expect AX values to biologically vary more widely over time than R5, R20, F_{res}, FEV_1 and FEF_{25-75} even in the absence of treatment modification. A post-hoc analysis assessing the effect of propranolol and salbutamol on spirometry and IOS measurements demonstrated that AX had the largest magnitude of response with respect to bronchoconstriction and bronchodilation compared to R5, F_{res}, FEV_1 and FEF_{25-75}.[225] Previously we have also shown that IOS has greater sensitivity than spirometry for detecting bronchodilator response using 400µg albuterol in asthma patients.[224]

The within-subject biological variability in ACQ was 0.6 units which is similar to the conventional MCID value of 0.5. Notably, the original paper by Juniper et al[115] studied patients with relatively well controlled asthma and a mean ACQ < 1.5. One could perhaps postulate that in our cohort of asthma patients with severe uncontrolled disease and a higher mean ACQ of 2.1, a higher CV and BV could be expected. Hence the 97.5%CI values presented for spirometry and IOS could perhaps be interpreted as the change that must occur for a clinically meaningful improvement in severe asthma patients. Importantly, our BV values for FEV_1 and FVC align with current American Thoracic Society (ATS) and ERS spirometry repeatability guidelines advising measurements within ≤150ml should be achieved between manoeuvres.[234]

One prospective trial investigating IOS variability in adolescent asthma patients demonstrated significant day-to-day differences in R5, R5-R15 and AX, but not spirometry in children who were maintained on a stable treatment regimen.[235] A recent prospective study observed moderate concordance between forced oscillation technique and spirometry values where the mean duration of time between measurements was 114 days in uncontrolled asthma patients taking a mean daily ICS dose of 1,015µg.[236] Another study[237] in clinically stable asthma patients found a moderate correlation between ACQ with spirometry and IOS measurements. We were therefore surprised that despite the majority of our patients undergoing a change in their ACQ score ≥0.5 no differences were observed in pulmonary

function between T1 and T2. Once again, this could perhaps reflect a slightly different disease pattern associated with severe asthmatics where there could be a disconnect between asthma control and lung function.

To our knowledge, this is the first study comparing medium term variability in impulse oscillometry and spirometry measurements over time in severe asthma. We appreciate the limitations of our study including the small sample size along with results from a single Scottish Centre and therefore larger studies with more serial longitudinal measurements are required to validate our results. We also appreciate there is a degree of uncertainty relating to disease control in our asthma patients over a relatively long duration (321 days) which could theoretically impact our results. Indeed, the wide range of intervals between the two evaluations is a significant limitation. However, the combination of no change in asthma therapy and no statistically significant or clinically relevant difference in FEV_1 between T1 and T2 might mitigate this possibility. One potential major limitation of our study was that patients were not precisely assessed between time point 1 and 2, and therefore this may be a source of possible bias. Although type 2 inflammatory biomarker results were only available in a subgroup of patients, PBE readings were intentionally averaged over the preceding 6 months due to significant temporal variability in severe asthma patients.[62]

In conclusion, we report on medium term repeatability for IOS and spirometry and propose values for within subject biological variability in patients with poorly controlled severe asthma.

Interactions between spirometry and oscillometry in patients with moderate to severe asthma

AIM: TO EVALUATE THE EFFECT OF COMBINING SPIROMETRY AND OSCILLOMETRY IN ASTHMA PATIENTS WITH REGARDS TO DISEASE CONTROL AND EXACERBATIONS

The small airways have previously been termed the quiet zone of the lungs as airways ≤2mm in diameter are traditionally more difficult to assess and treat in asthma.[238] The small airways are of particular interest to clinicians due to its close association with type 2 inflammation and asthma control.[79]

Spirometry involving a forced expiratory manoeuvre plays a pivotal role in the assessment of asthma although current Global Initiative for Asthma guidelines do not emphasise its role in measuring small airways dysfunction using forced expiratory flow rate between 25 and 75% of forced vital capacity (FEF_{25-75}). Moreover impaired FEF_{25-75} has been shown to be a sensitive marker of small airways disease in asthma.[155] Impaired FEF_{25-75} is associated with airway hyperresponsiveness, greater rates of healthcare utilisation, higher fractional exhaled nitric oxide (FeNO) and sputum eosinophils.[239,240]

Respiratory oscillometry involving effort independent tidal breathing has conventionally been used in clinical research, paediatric medicine and for adult patients unable to generate the necessary expiratory flow rate required for spirometry testing.[171] Resistance heterogeneity measured between 5 and 20Hz (R5-R20) reflects peripheral airway resistance and is highly concordant with small airways narrowing.[158] A recent large prospective study eloquently demonstrated the utility of oscillometry measurements reflecting small airways dysfunction across GINA asthma severities including lung reactance measured either at 5Hz (X5) or as area under the reactance curve (AX), as well as R5-R20.[155]

A systematic review of physiological tests for detecting small airways dysfunction including FEF_{25-75} and oscillometry for the diagnosis of asthma was inconclusive in determining the most useful modality.[241] Instead of an individual gold standard pulmonary function test, we postulate whether combining spirometry and oscillometry measurements of small airways function will be the way forward for optimal phenotyping of adult asthma patients. We aim to evaluate the interaction between spirometry and oscillometry defined small airways

function using FEF_{25-75} as a starting point. Therefore we compared spirometry, oscillometry, type 2 biomarkers, severe exacerbations and asthma control between: (a) patients with impaired FEF_{25-75} in conjunction with preserved or impaired oscillometry, (b) patients with preserved FEF_{25-75} in conjunction with preserved or impaired oscillometry; using cut offs of 60% for FEF_{25-75} and 0.10kPa/L/s for R5-R20.[7]

Data from 154 respiratory physician diagnosed moderate-to-severe asthma patients were retrospectively collected from patients attending either the National Health Service specialist asthma clinic or during a screening visit for a prior clinical trial in the Scottish Centre for Respiratory Research. Notably, patients with other respiratory conditions including chronic obstructive pulmonary disease and bronchiectasis were excluded from this study. Patients were divided into four groups based on the interaction between their spirometry and oscillometry small airway function: (a) preserved FEF_{25-75} with preserved oscillometry: $FEF_{25-75}\geq60\%$, R5-R20<0.10kPa/Ls (b) preserved FEF_{25-75} with impaired oscillometry: $FEF_{25-75}\geq60\%$, R5-R20≥0.10kPa/L/s (c) impaired FEF_{25-75} with preserved oscillometry: $FEF_{25-75}<60\%$, R5-R20<0.10kPa/L/s (d) impaired FEF_{25-75} with impaired oscillometry: $FEF_{25-75}<60\%$, R5-R20≥0.10kPa/L/s.

FeNO was measured using NIOX VERO (Circassia, Oxford, UK) according to the manufacturer's instructions and ATS guidelines. Spirometry (Micromedical, Chatham, UK) was performed according to ERS/ATS guidelines. Oscillometry was measured using IOS Masterscreen (Carefusion Hoechberg, Germany). Measurements were performed in triplicate to assess oscillometry according to the ERS technical standards with oscillometry always performed prior to spirometry. Accuracy of resistance measurements was confirmed on each day with a 3L calibration syringe (Masterscreen) and verified with the manufacturer's reference resistance device (0.2kPa/L/s).

Blood testing was performed for peripheral blood eosinophils and total IgE. Asthma control was determined using the 6-point asthma control questionnaire (ACQ), and the number of OCS-requiring asthma exacerbations in the preceding year were obtained from medical records.

Statistical analysis was performed using SPSS version 27. Data were assessed for outliers and for normality with Shapiro-Wilks prior to analysis. An overall analysis of variance was

performed to evaluate any significant differences in spirometry and ACQ (mean, 95% CI) between the four groups followed by pairwise comparisons (group a vs b and group c vs d) with Bonferroni correction and a two tailed alpha error set at 0.05. Significant comparisons for oscillometry and OCS exacerbations (median, IQR) were performed using independent samples Kruskal-Wallis tests. A small amount of data for X5, AX and Fres were unfortunately unavailable following interrogation of the oscillometry system. Additionally, to avoid over-investigation, not every patient had blood testing in cases where results were unlikely to change management. For missing data, analyses were performed with the number of data points stated in table 11. For National Health Service patients, Caldicott approval was obtained whilst for clinical trial patients informed consent and ethical approval was obtained via the East of Scotland research ethics service prior to data collection.

Mean (SEM) overall demographic data are shown in table 10.

Table 10 *Overall patient demographics*

Gender (F/M)	102/52	**OAH (%)**	47
Age (yrs)	50 (1)	**Anti-IL4rα (%)**	3
BMI (kg/m^2)	31 (0.6)	**Anti-IL5rα (%)**	21
LABA (%)	82	**FEV_1%**	86 (2)
LAMA (%)	45	**Ex-smoker (%)**	19
LTRA (%)	51	**ICS dose (µg)**	1594 (41)
THEO (%)	19		

Mean (SEM)

ACQ scores were significantly higher indicating worse control in conjunction with more frequent exacerbations in patients who exhibited combined impairment of FEF_{25-75} and R5-R20, while there were no differences in peripheral blood eosinophils or total IgE (Table 11). Patients with combined impairment of both FEF_{25-75} and R5-R20 also had significantly lower FEV_1, FEF_{25-75}, FVC, the latter indicating increased air trapping.

Pointedly, those with impaired spirometry as FEF_{25-75} and impaired oscillometry as R5-R20 had significantly worse asthma control as a 1.0-unit difference in ACQ and more exacerbations requiring oral corticosteroids than those with impaired FEF_{25-75} but preserved R5-20. The presence of impaired peripheral flow and resistance was not however associated with altered peripheral blood eosinophils or total IgE. The absolute difference in ACQ score was 1.0 which

exceeded the minimal clinically important difference of 0.5 units. Previously it has been shown that each 1.0 point increase in ACQ score is associated with a 50% increased exacerbation risk in moderate to severe asthmatics.[242] In other words, the results with regards to ACQ and exacerbations point to the findings being clinical meaningful. Indeed, a previous health informatics study in mild to moderate asthma patients showed that combined impairment of spirometry and oscillometry as FEF_{25-75} and R5-R20 respectively showed significantly worse asthma control defined by increased oral corticosteroid and short acting beta agonist use over 2 years.[220]

Table 11 *Significant differences in spirometry, oscillometry, type 2 biomarkers, asthma control and OCS requiring exacerbations comparing FEF_{25-75} ≥60%, R5-R20 <0.10kPa/L/s versus FEF_{25-75} ≥60%, R5-R20 ≥0.10kPa/L/s; and FEF_{25-75} <60%, R5-R20 <0.10kPa/L/s versus FEF_{25-75} <60%, R5-R20 ≥0.10kPa/L/s*

	FEF_{25-75}≥60%		FEF_{25-75}<60%	
	R5-R20 <0.1	R5-R20 ≥0.1	R5-R20 <0.1	R5-R20 ≥0.1
FEV_1 (L)	3.24 (3.05 – 3.43) (n=40)	2.71 (2.48 – 2.95) ** (n=22)	2.45 (2.20 – 2.69) (n=34)	1.91 (1.75 – 2.06) *** (n=58)
FEV_1 (%)	105.2 (100.5 – 109.9) (n=40)	97.0 (92.1 – 101.9) * (n=22)	79.6 (75.0 – 84.3) (n=34)	73.1 (68.1 – 78.2) (n=58)
FEF_{25-75} (L/s)	3.17 (2.88 – 3.46) (n=40)	2.62 (2.35 – 2.88 * (n=22)	1.35 (1.13 – 1.57) (n=34)	1.09 (0.97 – 1.21) † (n=58)
FVC (L)	4.02 (3.77 – 4.26) (n=40)	3.37 (3.06 – 3.67) ** (n=22)	3.90 (3.53 – 4.27) (n=34)	3.05 (2.81 – 3.29) *** (n=58)
FVC (%)	109.6 (104.7 – 114.5) (n=40)	101.6 (95.5 – 107.7) (n=22)	104.2 (99.1 – 109.4) (n=34)	96.2 (90.9 – 101.5) (n=58)
FEV_1/FVC	80.9 (79.2 – 82.6) (n=40)	80.8 (78.8 – 82.7) (n=22)	63.8 (60.4 – 67.3) (n=34)	63.8 (60.9 – 66.8) (n=58)
R5 (kPa/L/s)	0.38 (0.14) (n=40)	0.55 (0.18) *** (n=22)	0.39 (0.15) (n=34)	0.74 (0.36) *** (n=58)
R20 (kPa/L/s)	0.34 (0.10) (n=37)	0.40 (0.16) (n=22)	0.35 (0.14) (n=34)	0.48 (0.21) *** (n=58)
R5-R20 (kPa/L/s)	0.06 (0.04) (n=37)	0.15 (0.07) *** (n=22)	0.05 (0.04) (n=34)	0.23 (0.24) *** (n=58)
X5 (kPa/L/s)	-0.10 (0.06) (n=30)	-0.17 (0.13) *** (n=17)	-0.12 (0.08) (n=31)	-0.30 (0.21) *** (n=44)
AX (kPa/L)	0.28 (0.32) (n=39)	1.13 (0.83) *** (n=22)	0.44 (0.46) (n=33)	2.58 (3.77) *** (n=56)
F_{res} (Hz)	11.41 (3.89) (n=39)	18.48 (5.04) *** (n=22)	13.40 (5.63) (n=33)	24.23 (8.14) *** (n=56)
FeNO (ppb)	15 (21) (n=33)	15 (19) (n=17)	29 (24) (n=28)	20 (17) * (n=42)
PBE (cells/μl)	225 (243) (n=38)	240 (314) (n=21)	350 (220) (n=29)	220 (328) (n=53)
Total IgE (kU/L)	64 (227) (n=35)	108 (306) (n=17)	105 (406) (n=25)	107 (370) (n=44)
ACQ	2.0 (1.5 – 2.5) (n=29)	2.2 (1.4 – 3.0) (n=21)	1.4 (1.0 – 1.9) (n=29)	2.4 (2.0 – 2.8) ** (n=48)
OCS exacerbations	1 (2) (n=37)	1 (4) (n=17)	1 (4) (n=25)	4 (3) * (n=42)

*†p=0.05 *p<0.05 **p<0.01 ***p<0.001, denotes Bonferroni corrected comparisons between groups for either FEF_{25-75} ≥60% or FEF_{25-75} <60%*
Values presented as arithmetic means (95%CI) except for oscillometry and OCS exacerbations where median (IQR) were used

Biological variability, a measurement of natural fluctuation over time, can be used as a surrogate for the minimal change that must be exceeded for a clinically significant treatment effect to occur.[5] The absolute differences in FEV_1 and FEF_{25-75} were 540ml and 260ml/s respectively between groups with impaired FEF_{25-75} with or without impaired R5-R20, which

exceeded the biological variability values in severe asthma amounting to 150ml for FEV_1 and 210ml/s for FEF_{25-75} (Table 11).[5]

Repeating the analysis using AX at a threshold of <1.0 or ≥1.0kPa/L yielded similar results (table 12).

In the present study, our overall cohort of uncontrolled moderate-to-severe asthma patients had a well preserved mean FEV_1 of 86% but impaired small airways function as evidenced by FEF_{25-75} of 54% and R5 of 169%. We appreciate the limitation of our study due to its retrospective nature, but we believe that these data emphasise the important synergistic effect of combining spirometry and oscillometry measurements as useful tools in identifying those with clinically relevant small airways dysfunction. Perhaps these results will lead current guidelines to adopt more widespread use of oscillometry as an important adjunct and the incorporation of small airways dysfunction as an additional treatable trait in the management of asthma in the near future.

Table 12 *Significant differences in spirometry, oscillometry, type 2 biomarkers, asthma control and OCS requiring exacerbations comparing FEF_{25-75} ≥60%, AX <1.0kPa/L versus FEF_{25-75} ≥60%, AX ≥1.0kPa/L and FEF_{25-75} <60%, AX <1.0kPa/L versus FEF_{25-75} <60%, AX ≥1.0kPa/L*

	FEF_{25-75}≥60%		FEF_{25-75}<60%	
	AX <1.0	**AX ≥1.0**	**AX <1.0**	**AX ≥1.0**
FEV_1 (L)	3.18 (3.00 – 3.36) (n=47)	2.67 (2.34 – 3.00) * (n=14)	2.47 (2.25 – 2.69) (n=39)	1.81 (1.65 – 1.97) *** (n=50)
FEV_1 (%)	104.8 (100.8 – 108.8) (n=47)	95.5 (88.8 – 102.1) * (n=14)	79.6 (75.1 – 84.1) (n=39)	72.0 (66.5 – 77.6) (n=50)
FEF_{25-75} (L/s)	3.11 (2.85 – 3.37) (n=47)	2.52 (2.22 – 2.82) * (n=14)	1.36 (1.17 – 1.56) (n=39)	1.03 (0.91 – 1.16) ** (n=50)
FVC (L)	3.94 (3.71 – 4.16) (n=47)	3.35 (2.90 – 3.81) * (n=14)	3.94 (3.61 – 4.28) (n=39)	2.92 (2.67 – 3.16) *** (n=50)
FVC (%)	109.4 (105.2 – 113.7) (n=47)	99.7 (91.8 – 107.5) (n=14)	103.7 (98.9 – 108.5) (n=39)	95.4 (89.5 – 101.3) (n=50)
FEV_1/FVC	80.9 (79.4 – 82.5) (n=47)	80.1 (77.7 – 82.5) (n=14)	63.7 (60.5 – 66.9) (n=39)	63.6 (60.3 – 66.9) (n=50)
R5 (kPa/L/s)	0.42 (0.39 – 0.46) (n=47)	0.58 (0.51 – 0.66) *** (n=14)	0.42 (0.38 – 0.45) (n=39)	0.79 (0.71 – 0.87) *** (n=50)
R20 (kPa/L/s)	0.38 (0.34 – 0.40) (n=44)	0.41 (0.33 – 0.48) (n=14)	0.36 (0.32 – 0.39) (n=39)	0.48 (0.44 – 0.52) *** (n=50)
R5-R20 (kPa/L/s)	0.06 (0.05 – 0.07) (n=44)	0.18 (0.15 – 0.21) *** (n=14)	0.05 (0.04 – 0.07) (n=39)	0.26 (0.22 – 0.31) *** (n=50)
X5 (kPa/L/s)	-0.11 (-0.13 – -0.10) (n=36)	-0.26 (-0.34 – -0.18) *** (n=11)	-0.13 (-0.14 – -0.11) (n=33)	-0.35 (-0.39 – -0.30) *** (n=41)
AX (kPa/L)	0.39 (0.32 – 0.47) (n=47)	1.64 (1.28 – 2.01) *** (n=14)	0.48 (0.40 – 0.56) (n=39)	3.82 (2.99 – 4.66) *** (n=50)
F_{res} (Hz)	12.56 (11.58 – 13.54) (n=47)	20.52 (19.33 – 21.70) *** (n=14)	13.68 (12.55 – 14.81) (n=39)	25.27 (23.72 – 26.83) *** (n=50)
FeNO (ppb)	17 (12 – 23) (n=37)	16 (10 – 24) (n=12)	30 (24 – 38) (n=30)	20 (15 – 26) (n=39)
PBE (cells/µl)	227 (178 – 291) (n=45)	198 (109 - 360) (n=13)	311 (238 – 407) (n=34)	233 (151 – 285) (n=47)
Total IgE (kU/L)	85 (49 – 146) (n=41)	73 (24 – 227) (n=10)	128 (71 – 231) (n=29)	117 (71 – 193) (n=37)
ACQ	2.0 (1.5 – 2.6) (n=37)	2.3 (1.4 – 3.2) (n=13)	1.4 (1.1 – 1.8) (n=31)	2.3 (2.0 – 2.7) ** (n=44)
OCS exacerbations	1.5 (0.9 – 2.1) (n=42)	1.9 (0.0 – 3.8) (n=9)	2.0 (1.3 – 2.8) (n=31)	3.1 (2.2 – 3.9) (n=34)

**p<0.05 **p<0.01 ***p<0.001, denotes Bonferroni corrected comparisons between groups for either FEF_{25-75} ≥60% or FEF_{25-75} <60%*
Values presented as arithmetic means (95%CI) except for R5-R20, FeNO, PBE and total IgE where geometric means (95%CI) were used

Chapter 4: The impact of nasal polyps on clinical phenotype in severe asthma

Introduction

Chronic rhinosinusitis (CRS) is one of the most common persistent conditions of the developed world, estimated to affect 5-12% of the general population, and requiring input from various specialists including primary care physicians, otolaryngologists, respiratory physicians and allergologists.[243,244] Current international guidelines recommend that diagnosis is made by clinicians on the basis of symptoms such as nasal blockage, discharge, facial pressure and loss of smell for at least 12 weeks.[244] Based on high quality evidence, medical therapy with long term nasal corticosteroids, short courses of oral corticosteroids and/or nasal irrigation with isotonic saline or Ringer's lactate are effective in treating CRS.[244] CRS with nasal polyps (CRSwNP) accounts for 18-20% of CRS.[245] Two studies have demonstrated efficacy of intravenous mepolizumab (anti-IL5) 750mg q4w in reducing the need for nasal polyp surgery and total polyp score.[246,247] Surgical management is usually indicated when CRSwNP is refractory to medical therapy.[244]

Asthma and CRSwNP are both characterised by similar pathophysiology and share the common type 2 inflammation cascade.[245] Those patients with concomitant asthma and CRSwNP have more difficult-to-control lower airway disease,[248,249] are more exacerbation prone, with increased airway obstruction and extensive eosinophilic inflammation than those with asthma alone.[250] Likewise, patients with both these comorbid conditions have increased rates of NP recurrence[251] and higher rates of corticosteroid dependence than those with NP alone.[252]

Recent phase 3 placebo controlled trials have evaluated type 2 biologics in the management of CRSwNP including anti-interleukin receptor 4 alpha (IL4rα) with dupilumab (SINUS 24 and SINUS 52 over 24 and 52 weeks), anti-IgE with omalizumab (POLYP 1 and POLYP 2 both over 24 weeks), anti-IL5 with mepolizumab (SYNAPSE over 52 weeks), and anti-IL5rα with benralizumab (OSTRO over 40 weeks), dupilumab and omalizumab having already been approved.[91,253-255] No head-to-head biologic trials have been performed yet but an indirect comparison of absolute and percentage improvements in nasal polyp score (NPS), the primary outcome, and 22-point sino-nasal outcome test (SNOT-22), a key secondary outcome from these trials is presented (table 13).

Table 13 *Phase 3 trials comparing type 2 biologics versus placebo where endoscopic nasal polyp score (NPS 0-8) was the co-primary end point and 22 item sino-nasal outcome test (SNOT-22 0-110) was a secondary outcome.*

Trial	**SYNAPSE**	**OSTRO**	**POLYP 1**	**POLYP 2**	**SINUS 24**	**SINUS 52**
Drug	MEPO	BENRA	OMA	OMA	DUPI	DUPI
Baseline NPS	5.5	6.14	6.25	6.25	5.94	6.09
Delta NPS	-0.8	-0.57	-1.14	-0.59	-2.06	-1.80
% change	15%	9%	18%	9%	35%	30%
Baseline SNOT-22	64	69	60	60	49	51
Delta SNOT-22	-13.7	-5.2	-16.1	-15.0	-21.1	-17.4
% change	21%	8%	27%	25%	43%	34%

Values are depicted for the absolute delta response from baseline with active drug vs placebo and for the % response (as delta/baseline). The absolute change in SNOT-22 exceeded the MCID of 8.9 with all biologics.

In light of the limitations of an indirect treatment comparison, one can perhaps still appreciate that the absolute percentage improvements in NPS and SNOT-22 were greater for dupilumab compared to the other biologics. One might speculate that a plausible reason for this differential response could be due to the upper airways being a major contributor of exhaled nitric oxide which in turn is regulated by IL13.[256] By blocking IL13, dupilumab halts trafficking of blood eosinophils into tissue thus potentially improving nasal polyp burden. Although all the aforementioned studies had comparable baseline biomarkers as blood eosinophils and total IgE, it would have been informative to have FeNO concentrations. Nevertheless, it is important to bear in mind that biomarkers could simply be a marker of disease in nasal polyposis rather than a therapeutic target.

Due to the aforementioned impact of concomitant CRSwNP on the severity of asthma and the differential effects of various biologics on CRSwNP, I performed a retrospective cohort study investigating the impact of CRSwNP on the clinical phenotype of patients with moderate to severe asthma.

The impact of nasal polyps on clinical phenotype in moderate to severe asthma patients

PRIMARY OUTCOME: DIFFERENCE IN TYPE 2 BIOMARKERS IN ASTHMA PATIENTS WITH NASAL POLYPS VERSUS THOSE WITH ASTHMA ALONE

SECONDARY OUTCOMES: DIFFERENCE IN SPIROMETRY, OSCILLOMETRY, ASTHMA CONTROL AND EXACERBATIONS IN ASTHMA PATIENTS WITH NASAL POLYPS VERSUS THOSE WITH ASTHMA ALONE

ABSTRACT

Background: Nasal polyps (NP) are a common comorbidity of asthma. Differences in disease endotype and phenotype may have treatment implications for these concomitant conditions, including biologic therapies.

Objective: To determine putative differences in type 2 biomarkers, lung function and asthma control in asthma patients with nasal polyps (AwNP) and those with asthma alone (A).

Methods: 140 consecutive moderate to severe asthma patients with or without endoscopic NP taking a daily inhaled corticosteroid (ICS) dose of ≥800µg and at least one second line controller were identified from our National Health Service specialist respiratory and rhinology clinics. Data were collected prior to starting on biologics including peripheral blood eosinophils (PBE), fractional exhaled nitric oxide (FeNO), allergy status, spirometry, impulse oscillometry (IOS), asthma control questionnaire, oral corticosteroid requiring asthma exacerbations, NP score and lund mackay (LM) score.

Results: PBE and FeNO were significantly higher ($p<0.01$) whilst specific and total IgE ($p<0.05$) were significantly lower in AwNP vs A. FeNO had sensitivity of 81% and specificity of 67% for detecting NP (AUC=0.76 $p=0.001$). AwNP patients had less severe asthma as reflected by fewer exacerbations ($p<0.001$), lower ICS dose ($p<0.001$) and less impairment of IOS ($p<0.05$).

Conclusion: Moderate to severe asthma patients with NP have higher levels of PBE and FeNO despite better asthma control and lower total and specific allergy than those without NP.

INTRODUCTION

Severe refractory type 2 (T2) asthma presents a significant challenge to physicians due to disease heterogeneity, heavy symptom burden, high healthcare consumption costs and concomitant T2 comorbidities.[1] Chronic rhinosinusitis with nasal polyps (CRSwNP) is a common comorbidity directly related to asthma severity,[257] with an approximate prevalence of 30% amongst severe refractory asthmatics.[258] Both conditions are thought to follow the common pathophysiological mechanism of T2 inflammation, typically characterised by increased cytokine expression of IL4, IL5 and IL13 in response to various triggers.[259]

Recently, clinicians have used T2 biomarkers such as peripheral blood eosinophils (PBE), fractional exhaled nitric oxide (FeNO) and allergic status (specific and total IgE) to classify severe asthma patients according to their underlying inflammatory endotype.[1] Eosinophilic proliferation, maturation, survival, activation and migration is governed by IL5, whilst IL13 is associated with FeNO as well as eosinophil tissue migration.[1]

The mainstay therapy for both CRSwNP and asthma consists of inflammatory suppression with local corticosteroids, followed by short courses of systemic corticosteroids for exacerbations.[257] CRSwNP patients refractory to medical therapy have traditionally been referred for consideration of functional endoscopic sinus surgery.[244] Promising results have been reported with anti-IgE, anti-IL5 and anti-IL4rα, proposed to target 'treatable traits' according to presence of T2 biomarkers in CRSwNP and asthma.[257] Although all three classes of biologics have resulted in significant improvements in key CRSwNP outcomes (NCT03085797) at the standard licensed subcutaneous doses used in concomitant asthma,[91,253] an indirect treatment comparison of omalizumab versus dupilumab in CRSwNP has demonstrated significantly greater improvements with the latter.[260] Notably, omalizumab and dupilumab are entering into mainstay therapy for the treatment of nasal polyps in the US, having been approved by the Food and Drug Administration. Nonetheless, further research is required to determine the impact of endotype on patient response to biologics with regard to NP.

In a recent study of severe asthma patients, PBE >420 cells/µl and FeNO ≥39ppb were found to be the best predictors of concomitant NP.[97] Furthermore, patients with allergic asthma and

concomitant allergic rhinitis (AR) exhibit higher levels of PBE and FeNO whilst having lower FEV_1% and FEF_{25-75}% compared to those with allergic asthma alone.[261] Although one study demonstrated that non-asthmatic patients with CRSwNP may have evidence of small airways dysfunction (SAD) measured by spirometry,[262] to our knowledge no studies have been performed to evaluate SAD with spirometry or impulse oscillometry (IOS) in asthma patients with CRSwNP. In contrast to spirometry, IOS involves a tidal breathing manoeuvre used to measure small airways function by assessing peripheral airway resistance as difference in resistance between 5 and 20 Hz (R5-R20), peripheral airway reactance as area under reactance curve (AX) and reactance at 5 Hz (X5), and resonance frequency (fres).

Therefore, we performed a retrospective analysis to identify putative differences in T2 biomarkers, lung function and asthma control in asthma patients with nasal polyps (AwNP) compared to those with asthma alone (A).

METHODS

140 consecutive moderate to severe asthma patients with or without endoscopic NP taking a daily beclomethasone diproprionate (BDP) equivalent inhaled corticosteroid (ICS) dose ≥800µg and at least one second line controller (LABA, LAMA, LTRA or theophylline) were identified from our National Health Service (NHS) specialist respiratory and rhinology clinics over a period of 3 years. CRS patients without endoscopic NP were excluded. Data on PBE, FeNO, allergic status, spirometry, IOS, asthma control questionnaire (ACQ), oral corticosteroid (OCS) requiring asthma exacerbations, nasal polyp score (NPS) and LM score were retrospectively collected. Values for all T2 biomarkers and lung function were taken prior to initiation of any biologics as these can affect PBE, FeNO and IgE. Values for PBE were taken as the mean of values over the previous year.

FeNO was measured using NIOX VERO (Circassia, Oxford, UK) according to manufacturer's instructions and ATS/ERS guidelines.[179] Blood testing was performed to detect presence of circulating levels of specific IgE antibodies to defined common allergens [Fluorescence enzyme linked immunoassay (Phadia Immunocap 250)]. In our NHS laboratory a specific IgE concentration greater than 0.35 kUA/L is considered a positive result. We characterised specific allergy for each patient either as: (a) number of positive specific IgE responses to

aeroallergens including cat, dog, silver birch, house dust mite and grass, and (b) mean specific IgE calculated as the sum of specific IgE in kUA/L divided by the number of aeroallergens tested.

Spirometry (Micromedical, Chatham, UK) was performed according to American Thoracic Society (ATS) and European Respiratory Society (ERS) guidelines.[170] Prior to attending the laboratory for spirometry, patients had been asked not to use their short acting beta-2 agonists for 6 hours, long acting beta-2 agonists and muscarinic antagonists, theophyllines and leukotriene receptor antagonists for 48 hours. IOS (Masterscreen, Carefusion Hoechberg, Germany) measurements were performed in triplicate according to the European Respiratory Society guidelines[171] with IOS always performed prior to spirometry. Accuracy of resistance measurements was confirmed on each day with a 3L calibration syringe and a standard 0.2 kPa/L/s resistance mesh. Nasal endoscopy (30° oblique rigid Hopkins 3.0 mm) was performed in our rhinology mega-clinic to obtain NPS with a maximum score of 8. Lund Mackay scores were calculated from the most recent CT scan to radiologically assess CRSwNP burden with a maximum score of 24. Patients with aspirin exacerbated respiratory disease (AERD) were identified through history.

Data were first analysed for normality using Boxplots. FeNO and specific IgE (kUA/L) values were logarithmically transformed prior to analysis to normalise their distribution. Receiver operating characteristic curves were plotted to determine pre-test probability of NP based on FeNO values. A chi-squared test was performed to compare the distribution of gender in each group. Independent Student's T-tests with alpha error set at 0.05 (2-tailed) were applied. Values are presented as arithmetic means (SEM) and geometric means (SEM) for FeNO and specific IgE.

Caldicott Guardian approval was obtained to allow access to any NHS patient identifiable data including allergy, PBE, FeNO, spirometry, IOS, ACQ, asthma exacerbations, NPS and LM score.

RESULTS

Table 14 depicts demographic data showing patients in the AwNP group were more likely to be male and older than those in the A group. 29/78 (37%) and 5/62 (8%) were taking maintenance OCS in the A and AwNP groups respectively. A subgroup of 63/140 (45%) also

had measurement of IOS. 27 (A, n=22; AwNP, n=5) and 55 (A, n=37; AwNP, n=18) patients subsequently commenced biologic treatment for asthma with either anti-IgE or anti-IL5 therapy respectively.

In the overall analysis (Table 14), PBE and FeNO were significantly higher whilst total and specific IgE were significantly lower in the AwNP group (Figure 6). Receiver operating characteristic (ROC) curves demonstrated that FeNO ≥22ppb had sensitivity and specificity values of 81% and 67% respectively for the association of NP (AUC=0.76, p=0.001) whilst PBE ≥324 cells/µl had sensitivity and specificity values of 80% and 46% respectively (AUC 0.67, p=0.003).

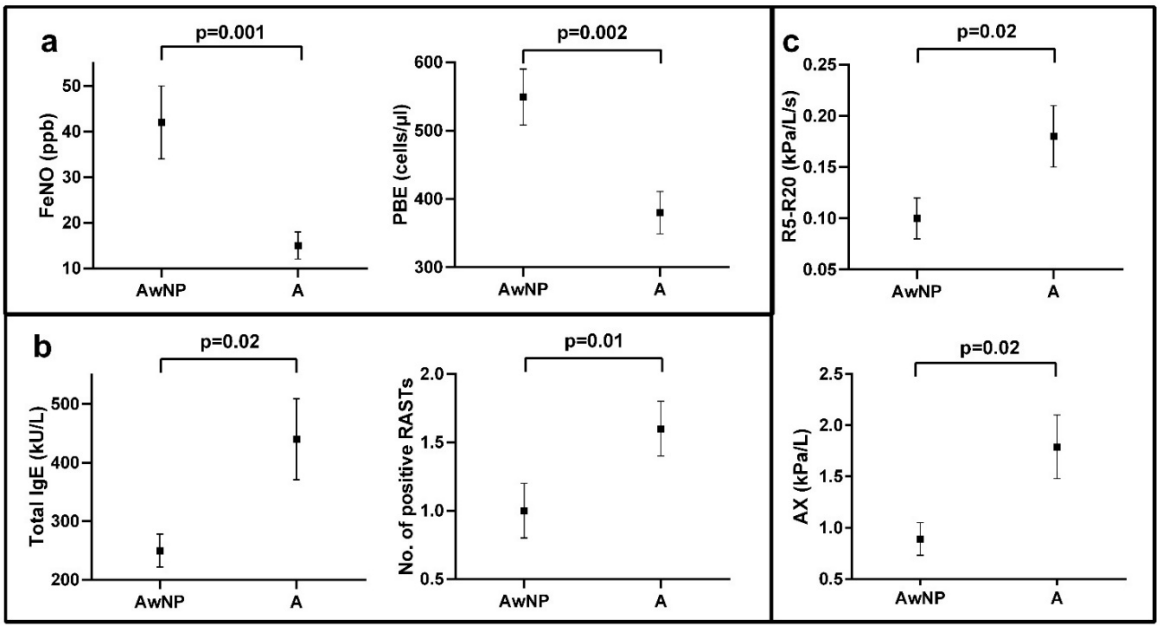

Figure 6 *Comparisons in type 2 biomarkers and oscillometry in asthma patients with or without NP (AwNP vs A) as arithmetic means or geometric (FeNO) and standard error of means, for significant comparisons in overall patient population according to (a) FeNO and PBE count (b) total IgE and number of positive specific IgE and (c) R5-R20 and AX*

AwNP patients had better asthma control as reflected by fewer exacerbations, a lower ICS dose and less impairment of IOS values (R5, R5-R20, X5 and AX). No significant differences in spirometry values were demonstrated between the two groups. The findings were similar when excluding the presence of allergic rhinitis (20/78) among asthma patients without NP (Table 15) and also among patients (AwNP, n=37; A, n=70) taking at least 1,500µg ICS.

However, in the sub-analysis of patients taking at least 1,500µg ICS, no significant differences in total or specific IgE were detected.

Table 14 *Demographic Data and Comparisons in Type 2 Biomarkers, Lung Function and Asthma Control*

	AwNP (SEM)	**A (SEM)**
Gender (F/M)	28/34	56/22 *
Age (yrs)	57(2)	51(2)
BMI (kg/m^2)	29(1)	31(1)
Ex-smokers (%)	37	26
Smokers (%)	3	5
LABA (%)	98	94
LAMA (%)	32	55
LTRA (%)	73	63
THEO (%)	11	36
INS (%)	100	26
INAH (%)	10	4
ICS dose (µg)	1,546(64)	1,892(44) ***
Maintenance OCS (%)	8	37
FEV_1%	84(3)	79(2)
FEF_{25-75}%	42(4)	46(3)
FVC%	106(3)	99(2)
FEV_1/FVC (%)	64(2)	68(2)
R5 (kPa/L/s)	0.45(0.03)	0.60(0.04) **
R5-R20 (kPa/L/s)	0.10(0.02)	0.18(0.03) *
X5 (kPa/L)	-0.14(0.02)	-0.23(0.03) *
AX (kPa/L)	0.89(0.16)	1.79(0.31) *
F_{res} (Hz)	15.59(1.07)	18.68(1.14)
PBE (cells/µl)	549(41)	380(31) **
FeNO (ppb)	42(8)	15(3) **
Specific IgE (kUA/L)	0.25(0.08)	0.84(0.34) *
No. of positive specific IgE	1(0.2)	1.6(0.2) *
Total IgE (kU/L)	250(28)	440(69) *
Asthma exacerbations	2(0.3)	4(0.4) ***
ACQ	2.5(0.3)	3.1(0.2)

ACQ = asthma control questionnaire; AR = allergic rhinitis; AX = area under reactance curve; AwNP = asthma with nasal polyps; A = asthma without nasal polyps; BMI = body mass index; exac = OCS requiring exacerbations; FeNO = fractional exhaled nitric oxide; FEV_1 = forced expiratory volume in 1 second; FEF_{25-75} = forced mid expiratory flow rate between 25 and 75% of forced vital capacity

(FVC); F_{res} = resonance frequency; ICS = inhaled corticosteroids; INAH = intranasal antihistamine; INS = intranasal corticosteroid; LABA = long acting beta agonist; LAMA = long acting muscarinic antagonist; LTRA = leukotriene receptor antagonist; PBE = peripheral blood eosinophils; R5 = resistance at 5Hz; R5-R20 = difference in resistance between 5 and 20Hz; THEO = theophylline; X5 = reactance at 5Hz; AwNP vs A p<0.05; p<0.01**; p<0.001***; FeNO and specific IgE are shown as geometric mean and SEM.*

Table 15 *Comparisons between groups according to use of high dose ICS and absence of allergic rhinitis in group A*

	ICS ≥1,500µg			
	AwNP (SEM)	**A (SEM)**	**AwNP (SEM)**	**A w/o AR (SEM)**
PBE (cells/µl)	541(53)	391(34) *	549(41)	387(34) **
FeNO (ppb)	41(9)	15(3) **	42(8)	16(4) **
Specific IgE (kUA/L)	0.25(0.1)	0.77 (0.34)	0.25(0.08)	1.05(0.48) **
No. of positive Sp IgE	1.1(0.2)	1.6(0.2)	1(0.2)	1.7(0.2) *
Total IgE (kU/L)	283(40)	467(76)	250(28)	462(81) *
FEV_1%	82(3)	80(2)	84(3)	81(2)
FEF_{25-75}%	40(4)	46(3)	42(4)	46(4)
FVC%	104(3)	99(2)	106(3)	101(2)
FEV_1/FVC (%)	64(2)	68(2)	64(2)	68(2)
R5 (kPa/L/s)	0.46(0.04)	0.62(0.04) *	0.45(0.03)	0.59(0.05) *
R5-R20 (kPa/L/s)	0.11(0.02)	0.18(0.03)	0.10(0.02)	0.18(0.03) *
X5 (kPa/L)	-0.14(0.03)	-0.24(0.03) *	-0.14(0.02)	-0.22(0.03) *
AX (kPa/L)	0.99(0.19)	1.81(0.30) *	0.89(0.15)	1.81(0.38) *
F_{res} (Hz)	15.81(1.29)	19.26(1.16)	15.59(1.07)	18.53(1.38)
Asthma exac	2.8(0.4)	4(0.3) *	2(0.3)	4(0.4) ***
ACQ	2.7(0.3)	3.2(0.2)	2.5(0.3)	3.1(0.2) *
ICS dose (µg)	1,943(24)	1,997(30)	1,546(64)	1,948(48) ***

No significant differences in T2 biomarkers, lung function or asthma control were demonstrated when comparing AwNP patients with aspirin exacerbated respiratory disease (AERD) (n=25) to AwNP patients without aspirin sensitivity (n=37). However, LM scores were significantly higher in AERD patients: 19(1) vs 15(1); p<0.01.

In the AwNP group, patients with a NPS ≥5/8 had significantly higher LM scores than those with NPS<5/8: 18(1) v 14(1); p<0.05. The overall mean LM score in the AwNP group was 16(1).

When excluding patients on maintenance OCS, PBE and FeNO values remained significantly higher in the AwNP group: 549(41) vs 393(31) $p<0.01$ for PBE and 44(9) vs 20(9) $p<0.05$ for FeNO.

DISCUSSION

Our patients without NP had worse asthma control in terms of more frequent exacerbations and associated higher ICS dose, which was mirrored by worse IOS but not spirometry. This is likely to reflect worse small airways dysfunction (SAD) defined by raised peripheral airway resistance (R5-R20) and peripheral airway reactance (AX or X5). Our data also showed that asthma control in more severe asthma patients is more closely related to SAD detected by IOS rather than spirometry, since FEF_{25-75} was not significantly different.[186] Although speculative, the significant differences in IOS measurements between the two groups could hypothetically be explained by the presence of two separate conditions on the same disease spectrum. It could be argued that one condition is characterised by inflammation of the nose, paranasal sinuses and larger airways whilst the other involves more distal portions of the bronchial tree. Further research is required to prove this theory and would provide more support for the incorporation of IOS into the standard work up for severe asthma.

In a recent large prospective study, the ATLANTIS group demonstrated that R5-R20 and AX measurements showed comparable prevalence of SAD in asthma patients at severities of GINA 1-3, a higher prevalence at GINA 4 and the highest prevalence at GINA 5.[155] Furthermore, one study aiming to validate the use of forced oscillation R5-R20 using computational models as a measure of small airway narrowing identified 0.08kPa/L/s as representing severe SAD.[158] However, more work is still required for the standardisation of IOS measurements and the establishment of normal ranges. A previous comparison between two forced oscillation devices, IOS Jaeger Masterscreen and airway oscillometry (AOS) Thorasys Tremoflo, has shown better agreement for small airways resistance rather than reactance, and that AOS may be more sensitive at measuring reactance in patients with airflow obstruction.[263]

The results of the present study also showed that the presence of NP in asthma patients was associated with higher PBE and FeNO, lower total and specific allergy burden, whilst asthma

control was better along with less small airways dysfunction. The higher PBE and FeNO in AwNP patients is perhaps expected as one might predict that two concomitant T2 conditions would be associated with a higher T2 burden than one alone.[1] According to ROC analysis, FeNO showed superior ability than PBE in determining the presence of NP in our cohort of asthma patients. FeNO levels were normal in our asthma patients without NP but previous literature shows that even a modest ICS dose, fluticasone proprionate 100µg/day, can produce a 52% FeNO suppression from baseline in mild to moderate asthma.[101] We took care to document PBE and FeNO values prior to patients commencing biologic therapy to avoid confounding. Average PBE counts over the preceding year were calculated as temporal variability in blood eosinophils is an important consideration.[62] In contrast, total and specific IgE were significantly lower in our patients with NP in line with a previous study.[252] Since dupilumab inhibits signalling of IL4 and IL13 suppressing both IgE and FeNO, this perhaps might in part explain why it is highly effective in treating both asthma and NP.[91]

As PBE count has previously been reported to be associated with worse IOS outcomes[185] we were somewhat surprised to find that IOS measurements were worse in our asthma patients without NP where PBE count was lower. Although ACQ was numerically lower in patients with NP, the difference of 0.6 between groups was not significant, which is somewhat surprising given that ACQ is a strong predictor of exacerbations.[242,264] As expected, our AwNP patients with AERD exhibited higher LM scores than those without aspirin sensitivity, reflecting a greater degree of underlying sinus inflammation in line with previous literature.[265]

Notably similar trends between groups were still observed when excluding group A patients without allergic rhinitis. We felt this was important to ascertain given patients with asthma and allergic rhinitis have worse lung function and higher T2 biomarkers than patients with asthma alone.[261]

We accept our study has several limitations. Firstly, our study was retrospective and did not look at serial changes over time, including the potential impact of instigating biologic therapy. In a five-year prospective follow-up study of 200 newly diagnosed asthma patients, accelerated decline in FEV_1 was associated with nasal polyps, PBE and FeNO.[266] Moreover, we have recently shown that biologic therapy improves IOS measurements in severe asthma patients with baseline SAD, where NP prevalence was comparable to previous literature estimates.[267] We do not believe that the limited sample size was relevant here as otherwise

we would have missed important differences in outcomes such as asthma exacerbations, ICS dose and IOS. Perhaps a larger sample size might have picked up commensurate differences in ACQ and spirometry, although we feel this is somewhat unlikely given the greater improvements in exacerbations compared to either ACQ or FEV_1 in T2 high asthma patients treated with biologics.[52,268] There may have been a confounding effect from differences observed in ICS dose since this is known to suppress FeNO and PBE levels.[269] However, in the sub-analysis of patients on ICS ≥1,500µg where no significant ICS dose difference was found, FeNO and PBE were still significantly higher in the AwNP group. Moreover, exclusion of patients on maintenance OCS also resulted in significantly higher PBE and FeNO levels in the AwNP group. Repeat analysis was also performed with the exclusion of current smokers, due to their association with suppressed FeNO, yielding similar results to the original analysis. We are also cognisant that type 2 biomarkers may simply reflect the presence of sinonasal inflammation,[256] and that single-breath humming has been used to differentiate nasal mucosal nitric oxide from sinus nitric oxide.[270]

The results of our study suggest that it may be worthwhile to consider investigating moderate to severe asthma patients with raised PBE and FeNO levels for sino-nasal disease with nasal endoscopy and CT imaging especially those with impaired sense of smell, as this may have subsequent treatment implications. In conclusion, moderate to severe asthma patients with NP have higher levels of PBE and FeNO but lower total and specific allergy than those without NP. Patients without NP had greater small airways dysfunction in association with worse control. Taken together this reinforces the importance of careful characterisation of endotype and phenotype in patients with moderate to severe asthma.

Chapter 5: Correlating radiological and clinical features in moderate to severe asthma

Introduction

High resolution CT (HRCT) scanning plays an important role in the diagnostic work up of severe asthma,[271] demonstrating utility in detecting abnormal radiological findings including bronchiectasis, bronchial wall dilatation, bronchial wall thickening, mucus plugging and emphysema.[272,273] However, a significant proportion of patients with difficult asthma do not receive HRCT scans. This was demonstrated in one real life study,[274] where difficult asthma patients who underwent HRCT scanning were significantly older with longer disease duration; had poorer spirometry-measured lung function; and were taking higher doses of inhaled corticosteroids.

This mimics our clinical experience in NHS Tayside where a significant proportion of patients pragmatically do not receive HRCT scanning due to the low likelihood of changing management and to reduce the risk of ionising radiation. Therefore, the aim of the following two studies were to correlate radiological and clinical features as well as to identify potential pulmonary function surrogates that could be used if HRCT scans were not available.

Clinical associations of mucus plugging in moderate to severe asthma

PRIMARY OUTCOME: DIFFERENCE IN SPIROMETRY, TYPE 2 BIOMARKERS AND SEVERE EXACERBATIONS IN PATIENTS WITH VERSUS PATIENTS WITHOUT MUCUS PLUGGING

SECONDARY OUTCOMES: DETERMINE ODDS RATIOS FOR CLINICAL ASSOCIATIONS WITH MUCUS PLUGGING; AND EVALUATE PREVALENCE OF MUCUS PLUGGING ACCORDING TO BRONCHOPULMONARY SEGMENT

ABSTRACT

Background: Mucus plugging (MP) is recognised as a contributory factor to airway obstruction and symptoms in persistent asthma.

Objective: We aimed to determine phenotypic associations of mucus plugging in patients with moderate-to-severe asthma in a real-life clinic setting.

Methods: MPs were identified by a thoracic radiologist on high resolution CT imaging. A MP score was subsequently calculated and analysed along with type 2 (T2) biomarkers, spirometry, severe exacerbations and asthma control for 126 moderate to severe asthma patients prior to biologic therapy.

Results: Asthma patients with MP had significantly worse FEV_1%, FEF_{25-75}% and FEV_1/FVC; higher levels of peripheral blood eosinophils, fractional exhaled nitric oxide, total IgE and A. fumigatus IgE titres; and experienced more frequent prior severe exacerbations. FEV_1/FVC, ≥2 exacerbations/yr, blood eosinophils, total IgE and A. fumigatus IgE titres were associated with MP after adjusting for confounders.

Conclusions: Poorly controlled asthma patients with MP exhibited significantly worse airflow obstruction, greater T2 inflammation associated with more frequent severe exacerbations. Impaired spirometry, more frequent exacerbations, raised blood eosinophils, total IgE and A. fumigatus IgE increased the likelihood of MP.

INTRODUCTION

Mucus plugging (MP), goblet cell hyperplasia, smooth muscle hypertrophy and eosinophilic infiltration are important pathophysiological characteristics of persistent asthma.[275] MP plays a significant contributory factor to airway obstruction and death in acute asthma[276] while its role in chronic asthma is also becoming better understood. One study demonstrated that 100% of 13 asthma patients with sputum eosinophils had evidence of MP on CT imaging compared to only 36% of 14 without sputum eosinophils.[108] Recently it has been shown that MP on high resolution computed tomography (HRCT) imaging in poorly controlled severe asthmatics is linked to exacerbations, airflow obstruction and type 2 inflammation albeit in different studies.[180,277]

Here we aim to determine clinically relevant associations of mucus plugging in patients with moderate-to-severe asthma. In particular we aim to pull together data on phenotypes including pulmonary function and type 2 (T2) biomarkers as well as asthma control and severe exacerbations requiring oral corticosteroids (OCS).

METHODS

126 respiratory physician diagnosed moderate-to-severe asthma patients attending the clinic between January 2016 and March 2022 taking a daily inhaled corticosteroid (ICS) dose of at least 800μg and a second line controller in line with the Global Initiative for Asthma guidelines were included in this retrospective cohort study (figure 7). Patients were included on the basis of pre-existing availability of HRCT scans with MP identified post hoc by an experienced thoracic radiologist using a recently published protocol.[180] Both lungs were assigned 10 segments each as follows: right upper lobe (3 segments); right middle lobe (2 segments); right lower lobe (5 segments); left upper lobe (3 segments); lingula (2 segments); left lower lobe (5 segments). The radiologist was blinded to all clinical data a priori except for the knowledge that patients had moderate-severe asthma. The HRCT scan was performed in volumetric mode with maximal inspiration, as per standard department protocol (128 slice CT Revolution EVO, GE Healthcare). CT reconstruction was performed in the lung window with a slice thickness of 1 - 1.25mm and no interval gap. Images were analysed in axial plane, with coronal and sagittal reconstruction used if necessary. MP was considered positive in this study if it

completely occluded the lumen of any order bronchus, and it was out with 2cm of the pleural surface. A mucus plug score (MPS) was subsequently calculated with 0 denoting no MP and a maximum score of 20 to signify all areas contained at least 1 MP.[180] HRCT scans were performed within 1 year of pulmonary function testing, T2 inflammatory markers and asthma control data. Peripheral blood eosinophils were averaged over the preceding year due to established temporal variability.[278] All measurements were taken prior to patients starting biologic therapy.

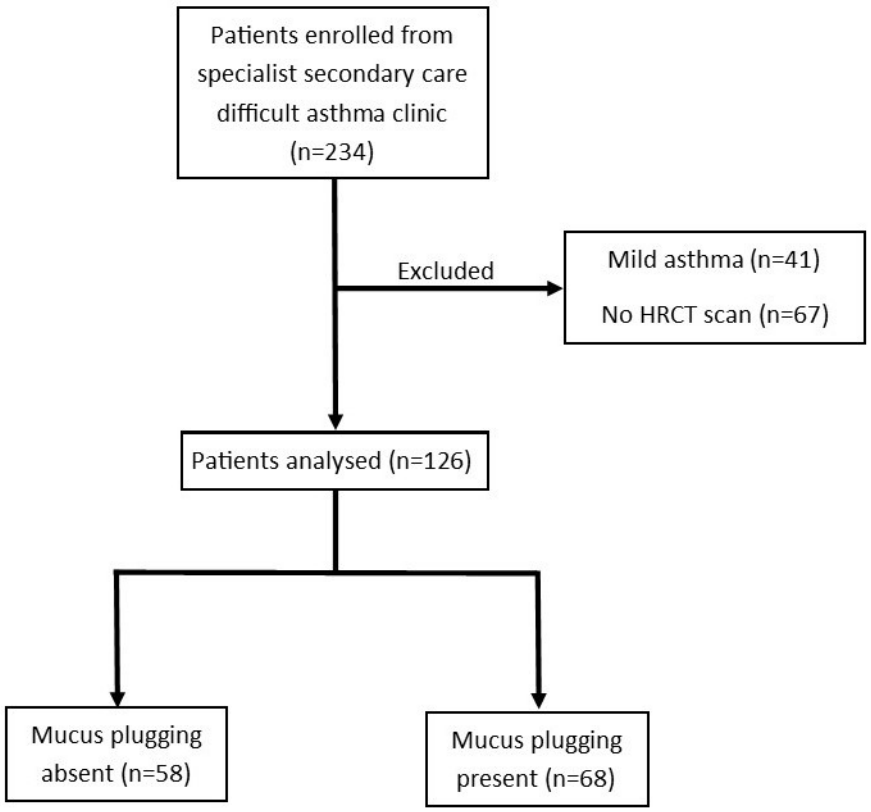

Figure 7 *Study flowchart*

FeNO was measured using NIOX VERO (Circassia, Oxford, UK) according to the manufacturer's instructions and ATS guidelines. Spirometry (Micromedical, Chatham, UK) was measured according to ERS/ATS guidelines. Blood testing was performed for total IgE, peripheral blood eosinophils (PBE) and IgG and IgE antibodies to Aspergillus fumigatus. Asthma control was determined using the 6-point Asthma Control Questionnaire (ACQ) and the number of oral

corticosteroid requiring exacerbations over the past 12 months prior to HRCT imaging was retrieved from medical records. The presence of nasal polyps and subsequent scoring using the Meltzer system[279] was determined by endoscopy (30° oblique rigid Hopkins 3.0 mm endoscope). The presence of bronchiectasis was based on the following criteria: non-tapering bronchus with an internal diameter 110% or greater than the adjacent pulmonary artery or the presence of visible bronchi within 1 cm of the costal pleural surface or adjacent to the mediastinal pleural surface.[280] Statistical analysis was performed using SPSS v27 with data assessed for outliers and for normality with histograms and Shapiro-Wilks prior to analysis. Independent T tests (normally distributed data) or Mann Whitney U tests were implemented to compare continuous variables between patients with or without MP using a two tailed alpha error set at 0.05. Logistic regression was implemented to calculate odds ratios (95%CIs) which were subsequently adjusted for age, gender, BMI, smoking history, presence of nasal polyps, long-acting beta agonist (LABA), long-acting muscarinic antagonist (LAMA) and leukotriene receptor antagonist (LTRA). Values are presented as mean (95%CI or SD) or median (IQR). Caldicott approval was obtained prior to any data collection.

RESULTS

Overall mean ± SD and median (IQR) patient characteristics are presented in table 16. 13 patients were taking maintenance OCS. In those with nasal polyps, mean ± SD nasal polyp and lund mackay scores were 6±2 and 16±5 respectively. The distribution of MPS is portrayed in figure 8.

Table 16 *Overall patient demographics*

Gender (F/M)	83/43	**Ex-smoker (%)**	25
Age (yrs)	52±14	**Current smoker (%)**	7
BMI (kg/m^2)	30.5±7.0	**FEV_1%**	77±23
LABA (%)	89	**FEF_{25-75}%**	49±28
LAMA (%)	57	**FVC%**	100±20
LTRA (%)	56	**FEV_1/FVC (%)**	68±13
THEO (%)	23	**ICS dose (µg)**	1740±421
OAH (%)	54	**FeNO (ppb)**	22 (29)
ACQ	2.4±1.4	**PBE (cells/µl)**	350 (285)
Exacerbations/yr	3 (4)	**Total IgE (kU/L)**	130 (356)
ABPA (%)	2	**Aspergillus IgE (kU/L)**	0.03 (0.19)
Bronchiectasis (%)	18	**Aspergillus IgG (mg/L)**	16.10 (22.42)
CRSwNP (%)	24		

Mean ±SD or median (IQR)

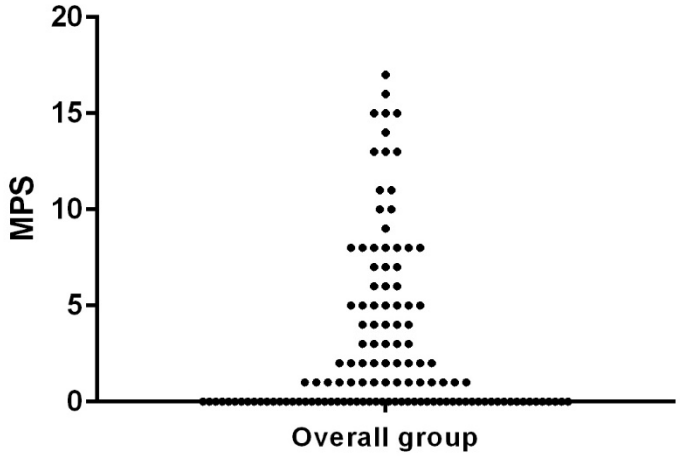

Figure 8 *Distribution of mucus plug scores in the overall moderate to severe asthma patient cohort (n=126)*

The overall median (IQR) MPS was 1 (5). Patients with MPS ≥1 had significantly lower FEV_1%, FEF_{25-75}% and FEV_1/FVC ratios but higher FeNO, peripheral blood eosinophils, total IgE and A. fumigatus IgE titres and significantly more frequent prior severe exacerbations (table 17). Those with MP were taking significantly higher ICS (BDP equivalent) doses (mean 1831 vs 1633µg, $p<0.01$). Repeating the analysis with bronchiectasis patients removed yielded broadly similar results (table 18). Table 19 presents the association between mucus plug score as the independent variable with spirometry, exacerbations and type 2 biomarkers as dependent variables using multiple linear regression.

The proportion of patients without versus with MP according to conventional pulmonary function and type 2 biomarker cut points are presented in table 17.

Table 17 *Comparisons in pulmonary function, type 2 biomarkers, asthma control and severe exacerbations between asthma patients with or without mucus plugging*

	MPS 0 (n=58)	**MPS ≥1** (n=68)
FEV_1 (%)	88.9±23.7	77.4±23.5 **
FEF_{25-75} (%)	59.4±29.7	39.6±23.5 ***
FVC (%)	101.9±19.1	99.3±19.9
FEV_1/FVC	0.73±0.12	0.64±0.13 ***
ACQ	2.5±1.4	2.3±1.3
FEV_1 <80%	29%	56%
FEF_{25-75} <60%	50%	84%
FEV_1/FVC <0.7	33%	66%
PBE ≥300 cells/µl	47%	73%
FeNO ≥25 ppb	37%	60%
Total IgE ≥100 kU/L	40%	68%
FeNO (ppb)	15 (24)	26 (34) *
PBE (cells/µl)	280 (300)	390 (248) **
Total IgE (kU/L)	83 (345)	190 (359) **
A. fumigatus IgG (mg/L)	15.35 (22.75)	18.25 (23.45)
A. fumigatus IgE (kU/L)	0.02 (0.04)	0.06 (0.83) ***
OCS Exac	1 (4)	4 (2) **

**p<0.05; **p<0.01; ***p<0.001. ACQ = asthma control questionnaire; FEF_{25-75} = forced expiratory flow rate between 25 and 75% of forced vital capacity (FVC); FeNO = fractional exhaled nitric oxide; FEV_1 = forced expiratory volume in 1 second; IgE = immunoglobulin E; IgG = immunoglobulin G; OCS = oral corticosteroid; MPS = mucus plug score. Spirometry and ACQ presented as means*±SD *whilst T2 biomarkers and exacerbations presented as median (IQR).*

Table 18 *Comparisons in pulmonary function, type 2 biomarkers, asthma control and severe exacerbations excluding bronchiectasis patients (n=103)*

	MPS 0 (n=57)	**MPS ≥1** (n=46)
FEV_1 (%)	88.3±23.5	74.0±22.6 **
FEF_{25-75} (%)	59.0±29.8	37.0±23.0 ***
FVC (%)	101.5±19.0	96.9±18.1
FEV_1/FVC	72.8±12.0	62.7±14.3 ***
ACQ	2.5±1.5	2.3±1.4
FeNO (ppb)	15 (24)	27 (27) *
PBE (cells/µl)	270 (293)	370 (293) †
Total IgE (kU/L)	87 (349)	196 (353) *
A. fumigatus IgG (mg/L)	15.50 (22.98)	18.25 (23.45)
A. fumigatus IgE (kU/L)	0.02 (0.05)	0.04 (0.53) *
OCS Exacerbations	1 (4)	4 (2) **

*†p=0.05; *p<0.05; **p<0.01; ***p<0.001. ACQ = asthma control questionnaire; FEF_{25-75} = forced expiratory flow rate between 25 and 75% of forced vital capacity (FVC); FeNO = fractional exhaled nitric oxide; FEV_1 = forced expiratory volume in 1 second; IgE = immunoglobulin E; IgG = immunoglobulin G; OCS = oral corticosteroid; MPS = mucus plug score. Spirometry and ACQ presented as means*±SD *whilst T2 biomarkers and exacerbations presented as median (IQR).*

Table 19 *Multiple linear regression analyses using spirometry, exacerbations and type 2 biomarkers as dependent variables and mucus plug score as the independent variable*

	MPS		
	Adjusted R^2	**Standardised beta coefficients**	**ANOVA P value**
FEV_1%	0.002	-0.084	NS
FEF_{25-75}%	0.108	-0.182	0.009
FEV_1/FVC	0.127	-0.169	0.004
Exacerbations	0.183	0.272	<0.001
PBE	0.139	-0.029	0.003
FeNO	0.182	0.115	0.002
Total IgE	0.022	0.142	NS
A. fumigatus IgE	-0.006	0.107	NS
A. fumigatus IgG	-0.035	0.068	NS

NS = non-significant

The likelihood of MP being present was significantly higher in association with reduced FEV_1/FVC ratio [OR 95%CI 3.01 (1.14, 7.97)], ≥2 exacerbations/yr [OR 95%CI 5.00 (1.55,

16.11)], raised PBE [OR 95%CI 3.23 (1.16, 8.96)], total IgE [OR 95%CI 3.20 (1.09, 9.37)] and A. fumigatus IgE titres [OR 95%CI 9.37 (1.82, 48.20)] (table 20). In an analysis of 2520 bronchopulmonary segments (126 patients x 20 segments) we identified the highest prevalence of MP in the right and left lower lobes (figure 9).

Table 20 *Odds ratios (95%CI) for spirometry, type 2 biomarkers and exacerbations in their association with the presence of mucus plugging score ≥1 using logistic regression modelling adjusted for confounding variables*

	MP	
	Crude OR (95%CI)	**Adjusted OR (95%CI)**
FEV_1 (44%)	3.06 (1.46, 6.41) **	2.45 (0.95, 6.31)
FEF_{25-75} (68%)	5.18 (2.27, 11.83) ***	2.64 (0.92, 7.55)
FEV_1/FVC (51%)	4.02 (1.91, 8.45) ***	3.01 (1.14, 7.97) *
Exac (61%)	4.58 (2.03, 10.31) ***	5.00 (1.55, 16.11) **
PBE (62%)	2.95 (1.37, 6.37) **	3.23 (1.16, 8.96) *
FeNO (48%)	2.30 (1.01, 5.27) *	2.09 (0.66, 6.56)
Total IgE (57%)	2.73 (1.29, 5.80) **	3.20 (1.09, 9.37) *
A. fumigatus IgE (22%)	3.91 (1.35, 11.33) *	9.37 (1.82, 48.20) **
A. fumigatus IgG (12%)	1.42 (0.45, 4.54)	1.99 (0.45, 8.79)

**p<0.05, **p<0.01; p<0.001. Adjusted for age, gender, BMI, ICS BDP, presence of nasal polyps, smoking, LABA, LAMA and LTRA. Cut points used: FEV_1 <80%; FEF_{25-75} <60%; FEV_1/FVC <0.7; Exac ≥2/yr; PBE ≥300 cells/µl; FeNO ≥25ppb; total IgE ≥100kU/l; A. fumigatus IgE ≥0.35kU/L and A. fumigatus IgG ≥40mg/L. % in brackets denotes proportion of patients with that specific phenotype.*

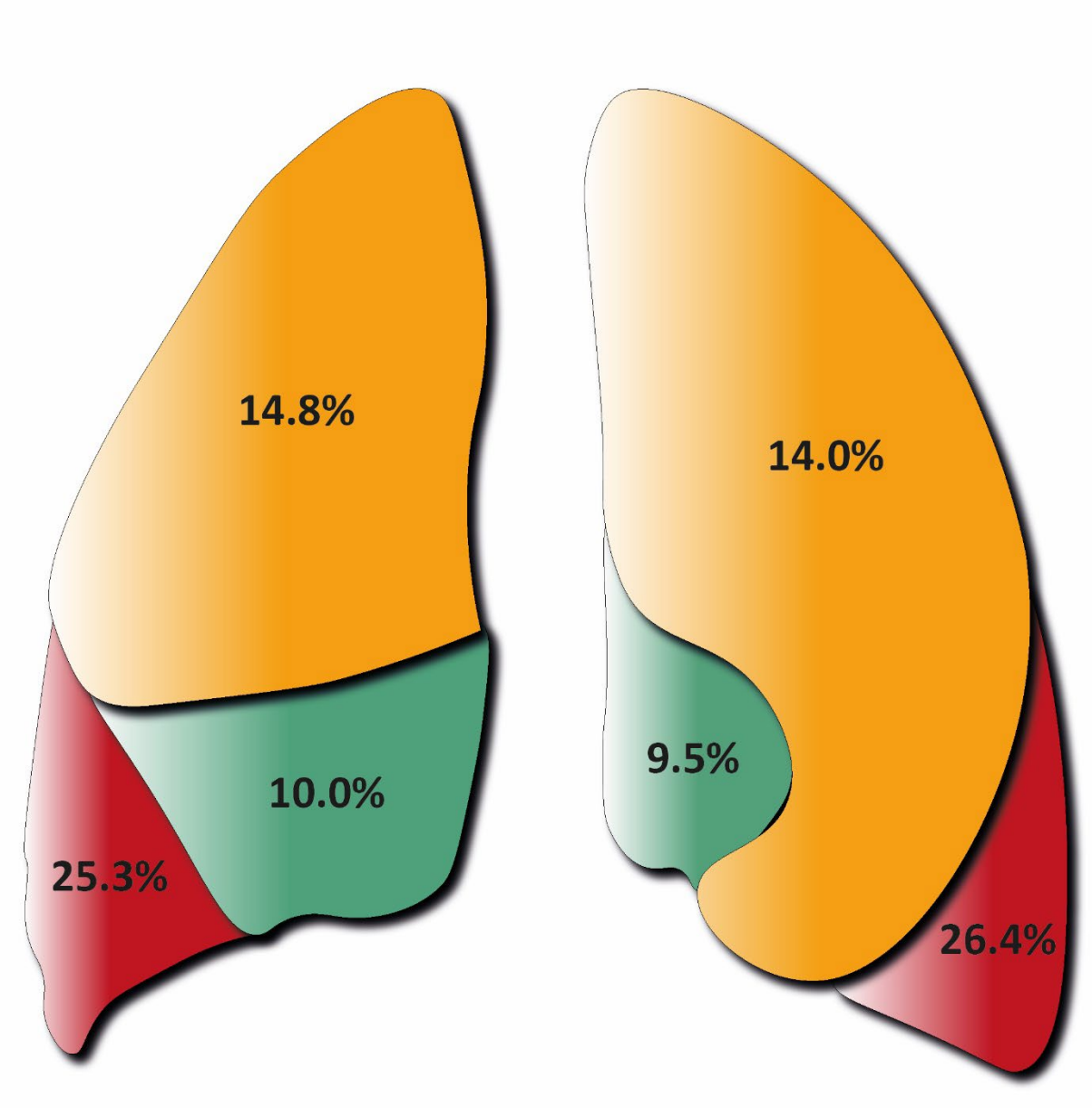

Figure 9 *Percentage prevalence of mucus plugging according to pulmonary lobe depicted by heatmap. Pulmonary lobes consistent with low (<10%), medium (10 – 15%) and high mucus plugging (>25%) are designated green, amber and red respectively.*

DISCUSSION

The main findings in the present study were twofold. Firstly, patients with MP had worse lung function, more frequent severe exacerbations requiring OCS and higher T2 biomarkers. Secondly, the presence of worse airflow obstruction, T2 inflammation and exacerbations were all associated with an increased likelihood of MP. For example, the adjusted odds ratios showed that the likelihood of MP was 67% higher in patients an impaired FEV_1/FVC, 80% higher in those with frequent exacerbations and 69% higher with raised PBE and IgE (figure 10). In contrast to previous studies,[180,277] our results are from a single UK centre. In our study, we opted to choose MPS as the outcome measure in an attempt to mimic real life clinical practice where most patients attending the severe asthma clinic would be expected to have had pulmonary function, blood tests and exacerbation history prior to CT imaging. In this regard, we have also included multiple regression analyses (table 19) where spirometry, type 2 biomarkers and exacerbations are the outcome measures.

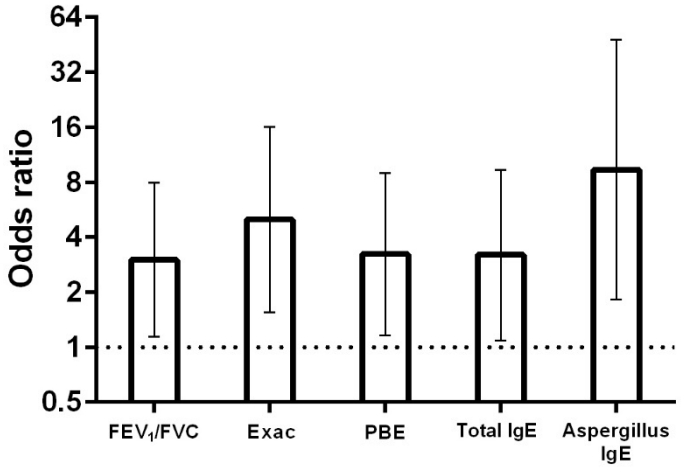

Figure 10 *Adjusted odds ratios (95%CI) for clinical outcomes associated with the presence of mucus plugging. Cut points used were: FEV_1/FVC <0.70; Exac ≥2/yr; PBE ≥300 cells/µl; total IgE ≥100kU/L; and aspergillus IgE ≥0.35kU/L. Interrupted line denotes level of unity that must be exceeded for a statistically significant effect.*

Notably, patients without MP had preserved FEV_1 (89%) and FEV_1/FVC (0.73) with the mean (95%CI) difference in FEV_1 between the two groups amounting to 401ml (91, 711), in keeping with a previous study linking an association between MP and airway obsrtruction.[180] FEF_{25-75} which is considered a surrogate for volume dependent small airways dysfunction,[181] was also significantly impaired in patients with MP with the mean (95%CI) difference being 0.80L/s (0.44, 1.17) aligning with a recent study.[281]

Mucus plugging is thought to be driven by IL4/13 mediated inflammation[108] and we therefore postulate that this is a potential mechanism by which the anti-IL4rα dupilumab may improve FEV_1 and FEF_{25-75} in severe asthma by reducing mucin and tissue eosinophils in addition to a direct effect on airway smooth muscle.[2] One study observed that a single dose of benralizumab, which depletes PBE, was also associated with improved regional lung ventilation along with reduced MP in turn inferring that eosinophils are involved in such plugs. In another study, although the presence of MP was not associated with a differential response to benralizumab in real life practice, this was notably in a homogenous patient cohort with no significant difference in T2 biomarkers between groups based on presence of MP.[282]

It has previously been demonstrated that asthma patients with higher MPS have significantly elevated sputum eosinophils, gene expression of IL13 and IL5 and MUC5AC/MUC5B ratios.[180] This explains our findings in this study that asthma patients with MP exhibit higher levels of routinely measured T2 biomarkers in real-life clinical practice including blood eosinophils, FeNO[283] and total IgE[281] with median values in the MP group all exceeding traditionally accepted cut points of ≥300 cells/µl, ≥25ppb and ≥100kU/L respectively. Furthermore, despite the suppressive effect of ICS on FeNO,[101] patients with MP were taking a significantly higher ICS dose but still had higher FeNO levels. We therefore postulate that asthma patients with the MP phenotype might potentially experience greater treatment response to biologics targeting their underlying inflammatory endotype.[1]

We also observed the greatest regional distribution of MP in the lower lobes in keeping with recent literature,[277] perhaps due to the effect of gravity on mucus secretions. Nevertheless, we were somewhat surprised that the right middle lobe and lingula had the lowest prevalence of MP given its frequent association with infection and bronchiectasis in other respiratory conditions.[284] In this study we used the ISHAM criteria[285] to diagnose allergic bronchopulmonary aspergillosis (ABPA). In turn it was perhaps intuitive that patients with MP

had higher median levels of specific IgE to A. fumigatus as mucus plugs are known to harbour fungal hyphae.[286] Recently it has been shown that airway eosinophils undergo cytolytic extracellular trap cell death and release filamentous chromatin fibres upon contact with A. fumigatus.[287] Furthermore, A. fumigatus also induces the MUC5AC gene resulting in increased mucus production by bronchial epithelial cells.[288] Additionally A. fumigatus sensitisation defined by a specific IgE ≥0.35kU/L was associated with an 89% increased risk of MP in this study, along with its historical associations with airflow limitation and bronchiectasis.[289] Interestingly, it has recently been shown that ABPA patients with mepolizumab-resistant bronchial MPs respond to benralizumab,[290] possibly related to the near complete blood eosinophil depletion with the latter.

We also demonstrated a significantly more frequent severe exacerbations requiring oral corticosteroids in patients with MP in keeping with a previous study.[277] This finding is clinically relevant as more frequent users of OCS have significantly increased morbidity and mortality.[291] We hypothesise that the lack of difference in asthma control can perhaps be explained by both groups having poorly controlled asthma with an overall mean ACQ of 2.4, and therefore the ability to detect differences may have been attenuated.

We recognise the main limitation of our study in terms of its retrospective observational nature. However, it has recently been shown that mucus plugs in 82% of asthma patients persist over 3 years potentially mitigating a time dependent effect.[277] There is a potential selection bias as patients were only included in this study if they had a previous HRCT scan. On the other hand, we hope that capturing results over a six-year period might alleviate this. Another potential limitation is that our study had one senior thoracic radiologist who interpreted the scans. Previous studies[180,277] have excluded current smokers or ex-smokers with ≥10 pack years. In contrast, we feel our study more mimics real life clinical practice as a significant minority of asthma patients are ex- or current smokers.[292] It is appreciated that the small airways are beyond the spatial resolution of HRCT and therefore MPS represent mucus plugging in the larger airways. Nonetheless the presence of a 20% difference in FEF_{25-75}% predicted might in turn point to the presence of worse small airway function in such patients. Furthermore, we appreciate that we only measured blood but not sputum or biopsy eosinophils because this was a real-life study where we do not routinely perform induced sputum or bronchial biopsy in the management of our asthma patients assessed in a busy

NHS clinic. The presence of mucus plugging should be recognised as a treatable trait for patients with severe asthma in terms of targeting therapy with biologics.

In conclusion, in a real-life clinic setting, the presence of mucus plugging detected on HRCT was associated with more severe exacerbations, more severe airflow obstruction and greater type 2 inflammation. This in turn suggests that imaging should be part of the routine work up of patients with poorly controlled severe asthma.

Impaired airway oscillometry is associated with bronchial wall thickening in persistent asthma

PRIMARY OUTCOME: TO DETERMINE THE CLINICAL ASSOCIATIONS WITH BRONCHIAL WALL THICKENING IN MODERATE-TO-SEVERE ASTHMA

ABSTRACT

Introduction: A recent study demonstrated a significant correlation between bronchial biopsy airway remodelling with quantitative computed tomography looking at bronchial wall thickness.

Methods: 92 respiratory physician diagnosed GINA-defined moderate-to-severe asthma patients were included in this retrospective cohort study. Blinded to all clinical data, two senior thoracic radiologists independently measured airway lumen and total airway area at four different bronchopulmonary segments using high resolution CT imaging. We calculated adjusted odds ratios (aORs) in regard to the association of bronchial wall thickness with spirometry, oscillometry, exacerbations, nasal polyps and type 2 biomarkers.

Results: The pooled analysis for all four bronchopulmonary segments showed that AX≥1.0kPa/L, R5-R20 ratio ≥25%, ≥2 exac/yr, nasal polyposis and PBE ≥300 cells/µl exhibited aOR (95%CI) of 3.54 (1.22,10.32); 2.89 (1.03,8.05); 4.17 (1.25,13.90); 9.85 (2.33,41.74); and 4.22 (1.44,12.38) respectively in their association with wall area thickness ≥50%. These translated into a respective 72%, 65%, 76%, 90% and 76% increased likelihood for wall area ≥50%.

Conclusion: Bronchial wall thickness is associated with peripheral airways resistance and reactance, severe exacerbations, nasal polyposis and peripheral blood eosinophilia in persistent asthma.

INTRODUCTION

Airway remodelling refers to structural changes to the bronchial wall in response to sustained unopposed asthmatic inflammation.[293] A recent study demonstrated a significant correlation between bronchial biopsy airway remodelling with quantitative computed tomography

looking at bronchial wall thickness.[294] Increased bronchial wall thickness and worse spirometry occurs in patients with more severe asthma patients.[295-297] However, to our knowledge, no studies have investigated the relationship between oscillometry and bronchial wall thickness in asthma.

METHODS

92 respiratory physician diagnosed GINA-defined moderate-to-severe asthma patients were included in this retrospective cohort study. Patients with COPD or bronchiectasis were excluded. Blinded to all clinical data, two senior thoracic radiologists independently measured airway lumen and total airway area at four different bronchopulmonary segments: right apical; right lower lobe posterior basal; left apico-posterior and left lower lobe posterior basal. Wall area percentage (WA%) measurements were subsequently calculated from these values. HRCT scans were performed in volumetric mode with maximal inspiration, as per standard department protocol (128 slice CT Revolution EVO, GE Healthcare). CT reconstruction was performed in the lung window with a slice thickness of 1-1.25mm and no interval gap. The images were analysed in multiplanar reconstruction and measurements done in a plane perpendicular to the corresponding segmental bronchi. Using free hand tool technique, the cross-sectional areas of airway lumen and total airway including wall were measured in mm^2. Two-way mixed intraclass correlation coefficients with absolute agreement were used to calculate reliability of airway lumen and total airway measurements: intra-observer and inter-observer ICCs (95%CI) amounted to 0.90(0.86,0.93) $p<0.001$ and 0.81(0.54,0.90) $p<0.001$ respectively.

Spirometry (Micromedical, Chatham, UK) was performed according to ERS/ATS guidelines. Oscillometry was measured using IOS Masterscreen (Carefusion Hoechberg, Germany) according to the ERS technical standards. HRCT scans were performed within 1 year of pulmonary function testing, exacerbation and asthma control data. All measurements were taken prior to patients starting biologic therapy. The presence of nasal polyps and subsequent scoring using the Meltzer system was determined by endoscopy (30° oblique rigid Hopkins 3.0 mm endoscope) with a max score of 8. Lund Mackay scores were calculated from the most recent CT scan to radiologically assess chronic rhinosinusitis with nasal polyposis (CRSwNP) burden with a maximum score of 24.

Statistical analysis was performed using SPSS v27. Data were assessed for outliers and for normality with Shapiro-Wilks prior to analysis. Logistic regression was performed to calculate odds ratios (95%CI) for predicting WA ≥50% and these were subsequently adjusted for age, gender, inhaled corticosteroid (ICS) dose, nasal polyps, smoking, body mass index (BMI), long-acting beta agonist (LABA) and long-acting muscarinic antagonist (LAMA). Pearsons correlation coefficients were also calculated to assess the heterogeneity of WA% measurements. Caldicott guardian approval was obtained prior to any data collection.

RESULTS

Mean (SEM) or median (IQR) demographic data are shown in table 21. When comparing patients according to asthma severity, severe asthmatics (n=72) required significantly higher ICS BDP equivalent doses (1942(17)µg vs 970(22)µg p<0.001) and had more frequent severe exacerbations requiring OCS (2.5(3) vs 0(4) p<0.05) than moderate asthma patients (n=20). In the patients with nasal polyposis (n=20), the mean (SEM) nasal polyp score and Lund Mackay score were 5.6 (0.3) and 16.1 (1.2) respectively.

Table 21 Overall patient demographics

Gender (F/M)	65/27	**Ex-smoker (%)**	25
Age (yrs)	50 (2)	**Current smoker (%)**	9
BMI (kg/m^2)	31.3 (0.7)	**CRSwNP (%)**	22
LABA (%)	89	**FEV_1%**	82.7 (2.5)
LAMA (%)	59	**R5-R20 (kPa/L/s)**	0.11 (0.23)
LTRA (%)	58	**AX (kPa/L)**	0.94 (2.85)
THEO (%)	25	**FeNO (ppb)**	25 (30)
OAH (%)	55	**PBE (cells/µl)**	330 (280)
ACQ	2.6 (0.2)	**Total IgE (kU/L)**	118 (338)
Exacerbations/yr	2 (4)	**ICS dose (µg)**	1730 (44)

All values as mean (SEM) except median (IQR) for oscillometry, exacerbations and type 2 biomarkers

The median (IQR) wall area thickness was 50(11)%. Adjusted odds ratios (95%CI) for spirometry, oscillometry, asthma control and exacerbations being associated with wall area thickness ≥50% are presented (table 22). In the pooled analysis of all four segments, R5-R20 ratio ≥25%, AX≥1.0kPa/L, nasal polyps, ≥2 exacerbations requiring OCS in the prior year and

PBE ≥300 cells/µl were significantly associated with a higher likelihood of WA ≥50% (figure 11). This amounted to a median difference (95%CI) of 1 (0,3) for exacerbations ($p<0.01$) and a geometric mean fold difference (95%CI) of -0.31 (-0.56, -0.05) for AX ($p<0.05$). WA% measurements for all individual bronchopulmonary segments demonstrated significant moderate correlations (all $p<0.001$) (table 23).

Table 22 *Adjusted odds ratios (95%CI) for spirometry, oscillometry, asthma control, nasal polyposis, exacerbations, and type 2 biomarkers associating with wall area thickness ≥50%*

	WA≥50%				
	Rt Apical	**RLL PB**	**Lt Apico-posterior**	**LLL PB**	**Pooled**
FEV_1 <80%	0.91 (0.23,3.62)	2.12 (0.77,5.88)	1.31 (0.50,3.47)	1.84 (0.69,4.91)	1.53 (0.57,4.12)
R5-R20 ratio ≥25%	1.63 (0.59,4.53)	3.16 (1.13,8.82) *	1.57 (0.58,4.24)	2.64 (0.99,7.03) †	2.89 (1.03,8.05) *
AX ≥1.0 kPa/L	1.15 (0.42,3.14)	4.05 (1.40,11.71) *	4.01 (1.32,12.18) *	4.11 (1.45,11.68) **	3.54 (1.22,10.32) *
ACQ ≥1.5	4.78 (1.18,19.34) *	2.40 (0.60,9.67)	2.30 (0.53,9.99)	5.49 (1.12,27.00) *	2.92 (0.67,12.80)
Sev Exac ≥2/yr	2.06 (0.67,6.32)	4.95 (1.39,17.65) *	5.47 (1.44,20.82) *	1.62 (0.52,5.05)	4.17 (1.25,13.90) *
Nasal polyps	8.51 (1.67,43.32) *	7.47 (1.73, 32.19) **	5.84 (1.73,19.72) **	4.28 (1.16,15.84) *	9.85 (2.33,41.74) **
PBE ≥300 cells/µl	1.95 (0.68,5.64)	2.34 (0.84,6.52)	2.62 (0.90,7.63)	2.26 (0.80,6.38)	4.22 (1.44,12.38) **
FeNO ≥25ppb	0.20 (0.05,0.80) *	1.19 (0.35,4.05)	1.25 (0.36,4.33)	0.71 (0.21,2.47)	0.96 (0.29,3.23)
Total IgE ≥100 kU/L	0.71 (0.23,2.24)	0.36 (0.12,1.11)	0.23 (0.07,0.77) *	0.57 (0.19,1.76)	0.37 (0.11,1.20)

†p=0.05 *p<0.05 **p<0.01
RLLPB (LLLPB): right (left) lower lobe posterior basal
WA≥50%: wall area thickness ≥50%
Sev exac: Severe exacerbations requiring oral corticosteroids

Table 23 *Pearson correlations for WA% measurements in relation to individual bronchopulmonary segments*

	Rt Apical	**RLL PB**	**Lt Apico-posterior**	**LLL PB**
Rt Apical		0.51***	0.57***	0.48***
RLL PB	0.51***		0.56***	0.58***
Lt Apico-posterior	0.57***	0.56***		0.64***
LLL PB	0.48***	0.58***	0.64***	

***p<0.001

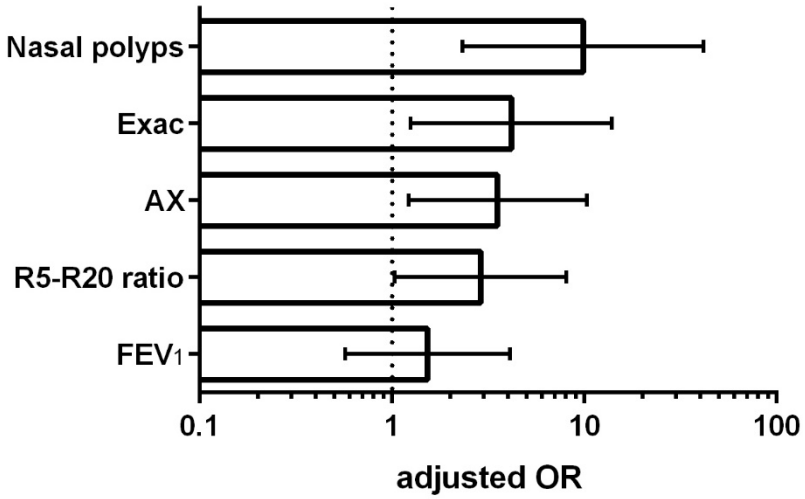

Figure 11 *Adjusted odds ratios (95%CI) for associations between bronchial wall thickness with FEV1, oscillometry, severe exacerbations and nasal polyposis*

DISCUSSION

In the present study we have demonstrated for the first time that peripheral airways resistance and reactance measured by resistance heterogeneity ratio (R5-R20 divided by R5) and reactance area (AX) respectively along with the presence of nasal polyposis and more frequent severe exacerbations requiring OCS are associated with increased bronchial wall thickness. The pooled analysis for all four segments showed that AX≥1.0kPa/L, R5-R20 ratio ≥25%, ≥2 exac/yr, nasal polyposis and PBE ≥300 cells/µl were associated with a 72%, 65%, 76%, 90% and 76% respective increased likelihood for WA≥50%. These results in turn suggest that oscillometry is perhaps a more sensitive tool for detecting airway remodelling than spirometry, especially in patients with more severe asthma where there is less disease heterogeneity. A R5-R20 ratio ≥20% is generally considered indicative of peripheral airways obstruction in adults[298] and here we used the pragmatic cut point of 25% as this was the closest reflection of the median value of 23% for our cohort. In this regard, AX is closely associated with poor control, worse quality of life, greater type 2 inflammation and more frequent severe exacerbations.[185,186,188] Whilst it has previously been shown that the severity of chronic rhinosinusitis is associated with bronchial wall thickening,[299] here we also demonstrate associations with nasal polyposis providing more evidence perhaps to support the unified airways disease concept.

As airway remodelling is primarily driven by bronchial inflammation, it was perhaps unsurprising that peripheral blood eosinophilia was significantly associated with greater airway wall thickness. A previous study examining the relationship between type 2 inflammation and airway wall thickness showed no correlation between eosinophil cationic protein and wall thickness.[295] The authors concluded that this was possibly confounded by inhaled corticosteroid use although it is also plausible that airway wall thickness from remodelling is a manifestation of an end-stage process from "burnt-out" asthma, similar to rheumatoid arthritis for example.[300] Furthermore, the authors obtained measurements from the right apical bronchopulmonary segment only, increasing the likelihood of sampling bias.[295]

No significant differences in asthma control as ACQ score were observed for pooled segments when comparing the two groups according to WA% although odds ratios were significant for right apical and left lower lobe segments on their own. We also found a disconnect between significant associations in AX but not R5-R20. This could be explained by R5-R20 reflecting a higher sensitivity to heterogenous narrowing of the smaller airways,[158] although it can also be affected by heterogeneity of the central airways and upper airway shunting.[301]

We appreciate our study has potential limitations including its retrospective nature arising from a real-life NHS clinic database. Firstly, the wide CIs for odds ratios were likely due to the limited sample size although significant associations were still detected. Secondly we evaluated more proximal generation bronchi as a surrogate for the distal airways as measuring the latter has traditionally been associated with difficulties in technique and repeatability.[302] Nevertheless, measurement of bronchial wall thickness is a time-consuming process that is unlikely to be performed on a routine basis in real life clinical practice. Instead, we now propose using AX as a potential surrogate for bronchial wall thickness and attendant airway remodelling. As opposed to measuring the right apical segment alone,[295] our study characterised bronchial wall thickening using pooled analyses from four segments with the aim to obviate any potential bias from sampling error. Pointedly, we excluded patients with bronchiectasis as bronchial dilatation would have likely resulted in an underestimation of wall area thickness. It would be interesting to repeat this analysis after long term follow up of patients who subsequently receive biologic therapy to assess the effects on airway remodelling.

Chapter 6: Effect of benralizumab on airway hyperresponsiveness in severe asthma

Efficacy of biologic therapy on airway hyperresponsiveness in persistent asthma

INTRODUCTION

Airway hyperresponsiveness (AHR) refers to an exaggerated bronchial response to a given inhaled agent and is governed by both a persistent structural element along with a variable inflammatory component in asthma.[303] This bronchial response is typically captured by measuring a drop in forced expiratory volume in 1 second (FEV_1), with the latter being associated with a more pronounced and steeper decrease at a smaller dose of constrictor agonist in severe asthmatics compared to those with mild or no asthma.[304]

Direct airway challenges using methacholine or histamine, which act directly on bronchial smooth muscle, can be used to assess AHR and are generally more sensitive in diagnosing asthma.[305] In this regard, methacholine has historically been preferentially used as histamine also acts on the bronchial sensory nerves and is less well tolerated for most patients.[306] Mannitol and adenosine monophosphate (AMP) are examples of indirect challenge agents which elicit endogenous AHR through the release of inflammatory mediators including prostaglandins, histamine and leukotrienes and are more specific to asthma.[307]

Airway inflammation drives AHR in asthma and this concept has previously been demonstrated by rapid improvements in indirect AHR following inhaled corticosteroid therapy in mild asthma.[308] In recent years, the advent of biologics[30] and anti-alarmins[2] have transformed severe asthma treatment in terms of reducing oral corticosteroid-requiring exacerbations and improving disease control, asthma quality of life and spirometry measured lung function. In contrast, there has comparatively been fewer studies investigating the efficacy of biologics in AHR and in this mini review, our aim is to summarise the existing evidence base in this area. Here, the objective is to obviate any potential confounding and therefore only clinical trials evaluating native AHR have been included and those testing allergen response excluded.

OMALIZUMAB (ANTI-IGE)

Effector mast cells are more abundant in the bronchial smooth muscle of asthmatic patients compared to those with eosinophilic bronchitis and controls, and are associated with greater AHR to methacholine.[309] Pointedly, immunoglobulin (Ig) E is responsible for activating mast cells and plays an important role in allergic asthma.[310]

Previous studies have investigated the effect of the anti-IgE monoclonal antibody omalizumab on AHR in patients with asthma. A randomised controlled trial (RCT)[311] (n=35) showed significant improvements in the provocative dose of acetylcholine required to drop FEV_1 by 20% (PC_{20}) amounting to a 0.42 mean doubling difference (dd) compared to placebo after 16 weeks of omalizumab in moderate-to-severe allergic asthma. Histamine release from basophils was significantly attenuated in the treatment group but interestingly, IL-13 and blood eosinophils were also significantly reduced with omalizumab.

However, in another RCT[312] (n=45) of patients with mild-to-moderate asthma, near-depletion of airway mucosal IgE and eosinophils were not accompanied by improvements in methacholine PC_{20} following 16 weeks of omalizumab. Only one RCT[313] in mild-to-moderate asthma used AHR as the primary outcome (n=34), but omalizumab did not improve methacholine or AMP PC_{20} compared to placebo after 12 weeks. Notably, the distinct difference of using methacholine instead of acetylcholine is the relative resistance to degradation by cholinesterase,[314] which in addition to disparities in asthma severity, may perhaps go towards explaining the difference in these results.

MEPOLIZUMAB (ANTI-IL5)

Bronchoalveolar lavage (BAL) eosinophil concentrations are greater in asthma patients with methacholine AHR,[315] and therefore one might postulate that airway eosinophil suppression with the anti-IL5 monoclonal antibody mepolizumab[316] would contribute to AHR attenuation. In patients with refractory asthma, one RCT[317] (n=61) did not detect any improvements in methacholine PC_{20} after 52 weeks of intravenous 750mg mepolizumab q4w. In another RCT[318] (n=24) looking at patients with mild asthma, the same dose of mepolizumab did not improve AHR measured by histamine PC_{20} after 20 weeks. Here, although blood and BAL eosinophils

were mostly suppressed, it is worth noting that 50% of airway tissue and bone marrow eosinophils were still remaining. This reservoir of eosinophils with ongoing degranulation as evidenced by persistence of major basic protein, could point towards a reason for the lack of efficacy in this study. Pointedly, the current licensed formulation for severe asthma is subcutaneous mepolizumab 100mg q4w and both studies did not utilise AHR as a primary outcome.

It has previously been shown that indirect AHR using AMP PC_{20} is more closely associated with airway inflammatory parameters such as sputum and blood eosinophils and eosinophil cationic protein than direct methacholine PC_{20}.[319] In this regard, I performed the recent phase IV clinical trial investigating the effect of anti-IL5Rα monoclonal antibody benralizumab 30mg q4w subcutaneously in severe asthma which is presented in its entirety later in this thesis.

TRALOKINUMAB (ANTI-IL13)

IL-13 has a multitude of key roles in the type 2 inflammatory pathway including its effect on smooth muscle hypertrophy, airway obstruction and AHR.[320] It was therefore surprising to see the results of one RCT in moderate-to-severe asthma[75] (n=79) where the anti-IL13 monoclonal antibody tralokinumab detected no significant change in methacholine PC_{20} after 12 weeks compared to placebo, especially as methacholine is more closely related to smooth muscle function and airway calibre than indirect challenge agents.[321] Despite reducing fractional exhaled nitric oxide (FeNO) and total IgE levels, blood eosinophil counts were increased with tralokinumab possibly due to reduced cellular trafficking from bronchial submucosa to blood, potentially explaining the lack of efficacy of tralokinumab. It is also worth mentioning here that methacholine AHR was not the primary outcome in this study. Intriguingly, one ex vivo study elicited AHR in the small airways with IL-13 and IL-4.[138] We therefore look forward to the results of the ongoing clinical trials investigating the effect of the anti-IL4Rα (blocking IL-4 and IL-13) monoclonal antibody dupilumab on mannitol (Eudract No. 2021-005593-25) and methacholine (NCT03884842) AHR.

TEZEPELUMAB (ANTI-TSLP)

Along with IL-25 and IL-33, the upstream epithelial alarmin thymic stromal lymphopoietin (TSLP) exerts its effect on downstream inflammatory cytokines IL-4, IL-5 and IL-13,[2] and therefore the results of the large genetic association study[322] showing an association between the TSLP gene variant with methacholine AHR is perhaps intuitive. More recently, two studies looking at the effect of the anti-TSLP tezepelumab on AHR measured by the provocative dose of mannitol required to drop FEV_1 by 15% (PD_{15}) have been published. In the first RCT[323] (n=40) looking at patients of any asthma severity, intravenous tezepelumab 700mg q4w did not improve mannitol PD_{15} as the primary outcome at week 12 compared to placebo although there was a strong trend (p=0.06). In this study, the mean blood eosinophil count was 214 cells/µl with attenuation of AHR most pronounced in patients with eosinophilic asthma. In the second RCT[129] (n=48) looking at patients with moderate-to-severe asthma and a mean blood eosinophil count of 287 cells/µl where AHR was an exploratory outcome, subcutaneous tezepelumab 210mg q4w significantly improved mannitol PD_{15} by a mean (95%CI) of 0.84 (0.04,1.65) dd after 28 weeks compared to placebo.

In addition to its broad spectrum effect on type 2 biomarkers,[2] tezepelumab may mechanistically improve AHR via its inhibitory effect on mast cell activation.[324] In this regard, it has been shown that the endogenous nucleoside adenosine is released by activated mast cells which in turn stimulates the release of effector cell mediators potentiating bronchoconstriction and AHR.[325] Preformed mast cell mediators include stem cell factor, histamine, adenosine and tryptase which have various autocrine and paracrine functions on eosinophils.[326] Other mediators are synthesized de novo and include IL5, granulocyte-macrophage colony stimulating factor, leukotriene C4 and prostaglandin D2. Conversely, activated eosinophils release eosinophil cationic protein and major basic protein that regulate mast cell function via MRGPRX2.[326]

SUMMARY AND OTHER CLINICAL CONSIDERATIONS

It is important to bear in mind that AHR is a continually moving target in that the presence and severity are dependent on choice of constrictor agonist and level of anti-asthma therapy. AHR is also the result of a complex biomechanisms that may have differential effects on

individual patients.[327] One health informatics study showed that 14% of methacholine responders were negative to mannitol, whereas 16% of mannitol responders were negative to methacholine.[328]

A graphical summary of the available biologic clinical trials in AHR are depicted (figure 12). Four of the nine clinical trials in this review included patients with mild asthma which probably would have resulted in a lower likelihood of detecting AHR attenuation since presumably there would be less room for improvement. With this review, we hope to highlight the urgent need for more biologic studies powered on AHR as the primary outcome although we duly appreciate the recent difficulties involving aerosol generating procedures in the pandemic era. Another potential area of research interest includes the contribution of the small airways to AHR since the small airways are more sensitive to bronchoconstriction in asthma.[225] In this regard, one study showed that asthma patients with spirometry-defined small airways obstruction had significantly greater AHR measured by histamine PC_{20}.[329] Future biologic AHR trials may benefit from the enhanced sensitivity of measurements of small airways obstruction such as oscillometry.

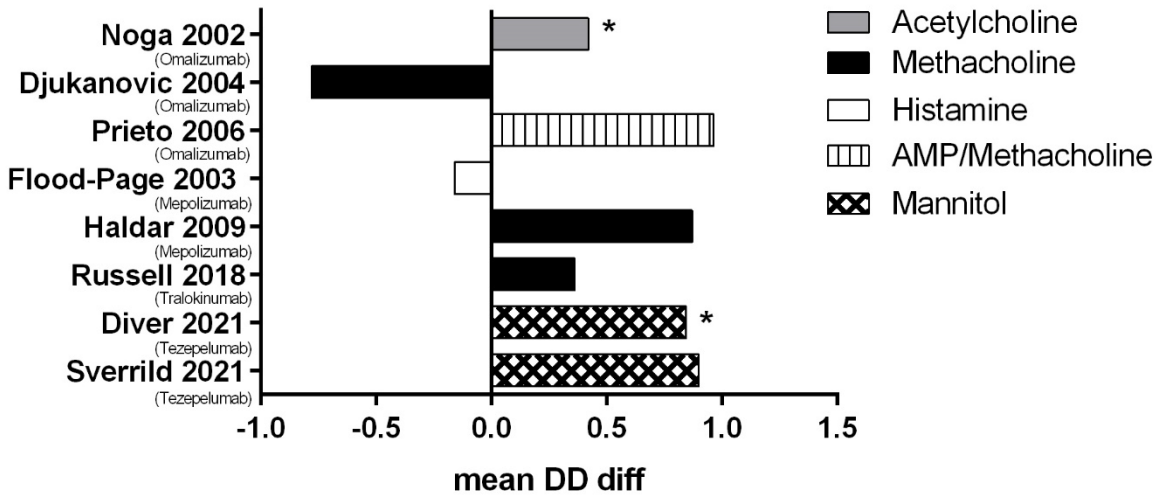

Figure 12 *Mean doubling dose difference in airway challenge agent for various biologics in asthma. *significant versus placebo.*

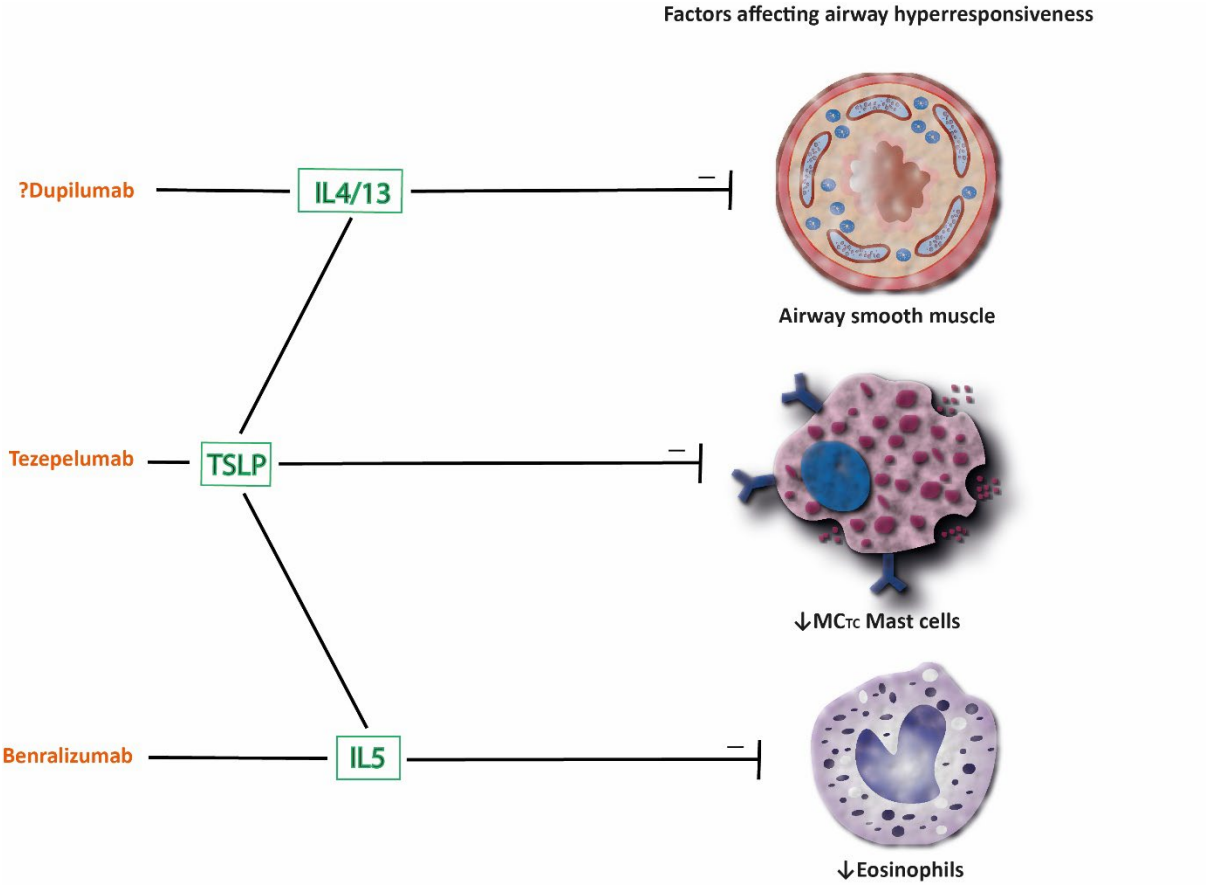

Figure 13 *Tezepelumab and benralizumab have demonstrated effectiveness in attenuating airway hyperresponsiveness in patients with persistent asthma through different pathways. Putative mechanism for dupilumab shown.*

Eosinophil depletion with benralizumab is associated with attenuated mannitol airway hyperresponsiveness in severe uncontrolled eosinophilic asthma

PRIMARY OUTCOME: TO DETERMINE THE EFFECT OF BENRALIZUMAB ON MANNITOL AIRWAY HYPERRESPONSIVENESS IN SEVERE EOSINOPHILIC ASTHMA

SECONDARY OUTCOMES: TO DETERMINE THE EFFECT OF BENRALIZUMAB ON ASTHMA CONTROL, ASTHMA QUALITY OF LIFE, DOMICILIARY PEAK FLOW, TYPE 2 BIOMARKERS, SPIROMETRY AND OSCILLOMETRY

ABSTRACT

Background: Airway hyperresponsiveness (AHR) and eosinophilia are hallmarks of persistent asthma.

Objective: We investigated whether eosinophil depletion with benralizumab might attenuate indirect mannitol AHR in severe uncontrolled asthma using a pragmatic open label design.

Methods: After a 4-week run-in period on usual ICS/LABA (baseline), adults with mannitol responsive uncontrolled severe eosinophilic asthma received 3 doses of open label benralizumab 30mg every 4 weeks followed by 16 weeks washout after the last dose. The primary outcome was doubling difference (DD) in mannitol PD_{10} at end point after 12 weeks, powered at 90% with n=18 patients required to detect 1 DD. Secondary outcomes included asthma control questionnaire (ACQ) and mini asthma quality of life questionnaire (mini-AQLQ).

Results: 21 patients completed 12 weeks with Benra at end point at week 12. Mean (SEM) age was 53 (4) years, FEV_1 80.2 (4.1) %, ICS dose 1895 (59)µg, n=12 on LAMA, n=13 on LTRA. Improvement in AHR was significant by 8 weeks with a mean 2.1 DD (95%CI 1.0, 3.3; $p<0.01$) change in PD_{10} at week 12, while mean changes in ACQ and mini-AQLQ were significant by week 2 and sustained over 12 weeks both exceeding the minimal important difference. Peripheral blood eosinophils were depleted by 2 weeks (439 to 6 cells/µl). No significant improvements occurred in lung function after 12 weeks. Domiciliary peak flow and symptoms were also improved with benralizumab.

Conclusion: Eosinophil depletion results in clinically meaningful attenuated AHR in severe uncontrolled asthma patients.

INTRODUCTION

Together with type 2 eosinophilic airway inflammation and variable airflow obstruction, airway hyperresponsiveness (AHR) plays a key role in the pathophysiology of severe asthma.[330] Indirect airway challenges such as mannitol measure AHR by promoting release of endogenous mediators from airway inflammatory cells resulting in bronchoconstriction.[331,332] As opposed to direct challenge such as methacholine or histamine acting on airway smooth muscle, indirect AHR is more closely associated with airway inflammation in patients with persistent asthma.[319] Indirect bronchial challenge test with mannitol identifies asthma with a high degree of specificity. [333]

The use of biologics has revolutionised the management of severe asthma, especially in relation to improvements in severe exacerbations, asthma control and quality of life.[1] Surprisingly there remains a paucity of data regarding the effect of biologics on AHR.

One study determined that IL-13 and IL-4, but not IL-5, induces histamine, carbachol and leukotriene D_4 mediated AHR in isolated human small airways.[138] However, another ex vivo study on passively sensitised human airways showed that the anti-IL5rα monoclonal antibody benralizumab was significantly more potent than anti-IL5 mepolizumab in attenuating direct AHR to histamine ex vivo.[334] Regardless, the effect of benralizumab on mannitol AHR in patients with severe eosinophilic asthma (SEA) is currently unknown. Therefore, we employed a pragmatic clinical trial design to investigate if eosinophil depletion due to benralizumab might attenuate indirect AHR with mannitol challenge as the primary outcome, with key secondary outcomes including asthma control and quality of life. In addition, we wished to see if such effects are maintained at 16 weeks after stopping benralizumab.

METHODS

Benralizumab in severe asthma (BISA) was a single arm open label phase 4 proof-of-concept clinical trial (EudraCT 2019-003763-22) that was conducted in the Scottish Centre for Respiratory Research between December 2020 and February 2022 primarily screening

patients with uncontrolled SEA. Eligible patients were those with an Asthma Control Questionnaire (ACQ) score ≥1.5, blood eosinophils ≥300 cells/µl, or the presence of CRSwNP or fixed airway obstruction and eosinophils ≥150 cells/µl, FEV_1 ≥50% and taking ICS/LABA at a dose ≥1000µg beclomethasone diproprionate equivalence. All patients screened in this study had a secondary care respiratory physician diagnosis of severe asthma according to GINA criteria (figure 15). Furthermore, those who were eligible for the study (n=21) all had their diagnosis verified by a positive mannitol bronchial challenge test[335] signified by the provocative dose required to decrease FEV_1 by 10% less than 635mg. Such patients entered into a 4-week run-in period on standard of care (baseline) and subsequently received 3 doses of subcutaneous benralizumab 30mg every 4 weeks in addition to standard of care over a 12-week treatment period followed by a washout period where no benralizumab was given for 16 weeks after the last dose (Figure 15).

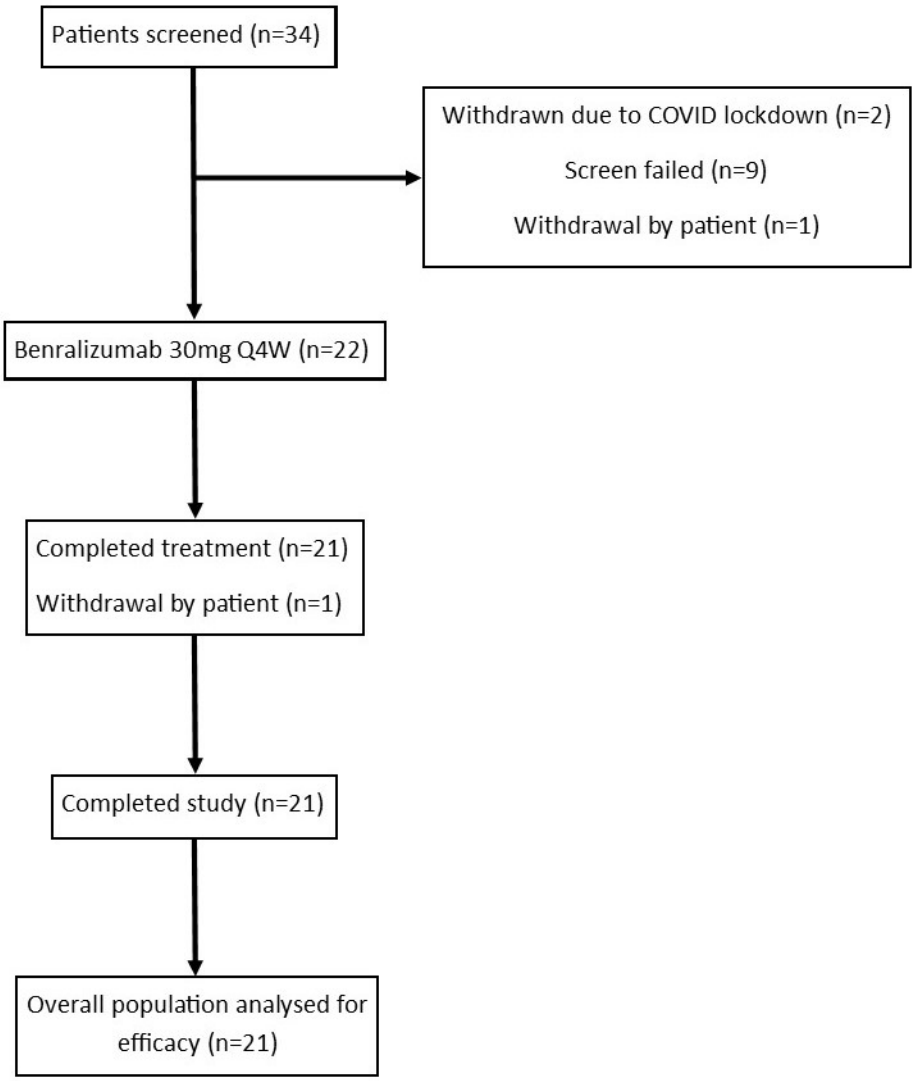

Figure 14 *Patient disposition*

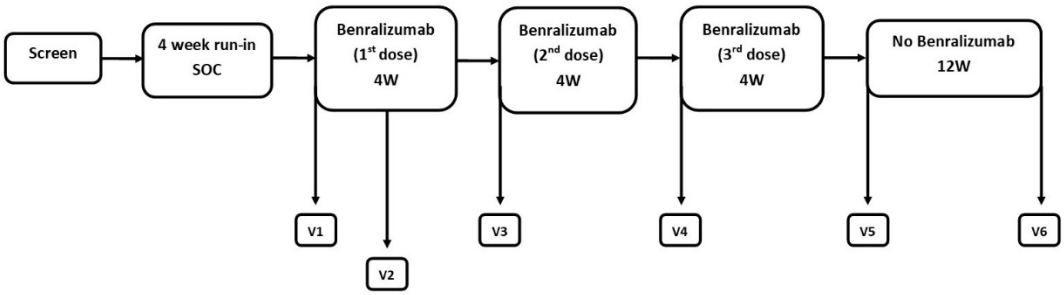

Figure 15 *Study flowchart*

The primary outcome was the doubling difference (log2 transformed) in the provocative dose of mannitol required to decrease FEV_1 by 10% (PD_{10}) after 12 weeks of benralizumab therapy. Although PD_{15} is usually used in clinical practice,[333] we elected to use the previously validated PD_{10} threshold[332,336,337] due to ethical concerns raised about the potential for severe bronchoconstriction in uncontrolled severe asthma patients. Bronchial challenges were performed using mannitol dry powder (Aridol, Pharmaxis, Sydney, Australia) as previously described.[332,336]

Key secondary outcomes included ACQ and mini-AQLQ. Other secondary outcomes of interest included peripheral blood eosinophils, eosinophil derived neurotoxin (EDN), fractional exhaled nitric oxide (FeNO), spirometry and oscillometry. FeNO was measured using NIOX VERO (Circassia, Oxford, UK) according to the manufacturer's instructions and ATS guidelines.[95] Spirometry (Micromedical, Chatham, UK) was performed according to ERS/ATS guidelines.[234] Oscillometry was measured using TremoFlo (Thorasys, Montreal, Canada) with measurements performed in triplicate according to the ERS technical standards with oscillometry always performed prior to spirometry.[338] Accuracy of resistance measurements was confirmed on each day with a standard 0.2 kPa/L/s resistance mesh.

As per aerosol generating procedural guidance for COVID-19, all bronchial challenges were performed whilst donning full personal protective equipment including a fluid resistant surgical gown, scrub cap, visor, FFP3 face mask and gloves. Using the supplied dry powder inhaler device, patients serially inhaled doubling doses: 0, 5, 10, 20, 40, 80, 160, 160 and 160mg of mannitol until a total cumulative of 635mg was attained. FEV_1 was measured 60 seconds after each inhalation, with the highest value of two recorded. The test ended once a

10% fall in FEV_1 was achieved, or when the maximum dose of 635mg had been given. The PD_{10} could then be calculated using log-linear interpolation of the dose response curve. Patients who did not achieve PD_{10} after the full protocol (i.e., PD_{10}≥635mg) had their values censored at 635mg for the purposes of statistical analysis. The within subject biological variability of mannitol AHR is +/-1 doubling dose shift such that values exceeding this shift from baseline in response to Benra were considered as being clinically relevant. Withholding times for asthmatic therapies prior to mannitol challenges were as follows: antihistamines, theophylline and LTRAs, 2 days; LABAs and LAMAs, 1 day; salbutamol or terbutaline, 6 hours. Patients were administered nebulised salbutamol 2.5mg immediately post-challenge to aid recovery.

Blood testing was performed for peripheral blood eosinophils and circulating levels of specific IgE antibodies with fluorescence enzyme linked immunoassay (Phadia Immunocap 250) to define common allergens including house dust mite, grass, cat, dog and silver birch. Serum eosinophil derived neurotoxin (EDN) levels were measured by commercially available ELISA kits (MBL Medical and Biological Laboratories 7630, Nagoya, Japan) for human EDN. All samples were systematically diluted by 1:5 when needed, and assayed following manufacturer instructions. The assay range after dilution was 3.0–200 ng/mL and the minimum detection limit was 0.62 ng/ml. Samples with an intra-assay coefficient of variation ≥15% were excluded from the analysis. Asthma control was determined using the 6-point Asthma Control Questionnaire (ACQ),[115] whilst quality of life was measured using the 15-point mini-Asthma Quality of Life Questionnaire (mini-AQLQ).[339] Patients were supplied study diary cards to document their daily reliever salbutamol use and domiciliary early morning peak expiratory flow (PEF) readings using a Mini-Wright peak flow meter (Clement Clarke, Harlow, UK) noting the best-of-three value. Patients were also asked to rate their early morning asthma symptoms using a 4-point nominal scale: 0, none; 1, mild; 2, moderate; and 3, severe. Reliever use, PEF and symptoms from the previous week were averaged for analysis. The MCID for PEF is 19 Lmin.[64]

Statistical analysis was performed using SSPS version 27 and graphs were prepared with GraphPad Prism 6 (GraphPad Software Inc). Data were assessed for outliers and for normality with normality plots and Shapiro-Wilks prior to analysis. An initial overall repeated measures analysis of variance (ANOVA) was performed to evaluate any significant differences between

the various timepoints. This was followed by Bonferroni corrected pairwise comparisons for each time point versus baseline as well as a separate comparison for week 12 vs 24, with a two tailed alpha error set at 0.05. Values are presented as arithmetic means (95% CI) except for PD_{10} and RDR as geometric means (95% CI). Ethical approval was obtained via the East of Scotland Research Ethics Service and written informed consent was taken from patients prior to any data collection.

RESULTS

Mean baseline demographic data are presented in table 24. Mean (95%CI) baseline bronchodilator reversibility was 270ml (179, 361) and 11.4% (8.3, 14.4) for FEV_1 whilst post-bronchodilator FEV_1/FVC ratio was 0.69 (0.64, 0.73). There were no significant differences comparing pre and post run-in (baseline) values for any outcomes (Table 25) although FeNO fell non significantly by 8ppb potentially due to improved ICS adherence during this period. However, mannitol challenge was only performed after the run-in.

Table 24 *Baseline patient demographics*

Gender (F/M)	9/12	**Ex-smoker (%)**	38
Age (yrs)	53 (4)	**Current Smoker (%)**	0
BMI (kg/m^2)	30 (1.2)	**Nasal polyps (Y/N)**	8/13
LABA (%)	95	**FEV_1%**	80.2 (4.1)
LAMA (%)	57	**FEF_{25-75}%**	41.5 (4.2)
LTRA (%)	62	**FVC%**	100.3 (3.9)
THEO (%)	14	**R5%**	161 (13)
OAH (%)	67	**ICS dose (µg)**	1895 (59)
CROMO (%)	5	**No. of +ve specific IgE**	2
INAH (%)	14	**Total IgE (kU/L)**	409 (180)
INS (%)	43	**OCS exac**	4 (2)

BMI = body mass index; CROMO = sodium cromoglicate; FEV_1 = forced expiratory volume in 1 second; FEF_{25-75} = forced mid expiratory flow rate between 25 and 75% of forced vital capacity (FVC); ICS = inhaled corticosteroid BDP equivalent dose; IgE = immunoglobulin type E; INAH = intranasal antihistamine; INS = intranasal steroid; LABA = long-acting beta agonist; LAMA = long-acting muscarinic antagonist; LTRA = leukotriene receptor antagonist; OAH = oral antihistamine; OCS = annual oral corticosteroid use; PBE = peripheral blood eosinophils; R5 = resistance at 5Hz; THEO = theophylline. Values presented as mean (SEM) except exacerbations presented as median (IQR).

Table 25 Mean differences, coefficients of variation and biological variability values between pre and post run-in (baseline). Mannitol PD_{10} was only performed after run-in at baseline. The biological variability value was calculated as the one sided 97.5%CI of the mean absolute change.

	Mean absolute change (95%CI)	Mean percentage change (95%CI)	Mean CV (95%CI)	Biological variability
ACQ-6	-0.3 (-0.7, 0.0)	-11.3% (-23.9, 1.3)	17.9% (9.1, 26.7)	0.4
Mini-AQLQ	0.2 (-0.2, 0.7)	7.0% (-5.8, 19.8)	14.1% (8.5, 19.8)	0.4
FEV_1 (L)	0.003 (-0.098, 0.105)	0.1% (-4.1, 4.4)	4.9% (3.5, 6.3)	0.102
FEF_{25-75} (L/s)	0.110 (-0.074, 0.294)	8.0% (-5.4, 21.4)	13.6% (8.9, 18.3)	0.184
FVC (L)	-0.056 (-0.168, 0.056)	-1.5% (-4.6, 1.5)	4.2% (2.7, 5.7)	0.112
FEV_1/FVC ratio	0.86 (-1.3, 3.0)	-1.3% (-2.0, 4.6)	4.6% (3.4, 5.8)	2.1
R5 (kPa/L/s)	0.00 (-0.04, 0.04)	0.6% (-7.1, 8.2)	9.4% (6.5, 12.4)	0.04
R20 (kPa/L/s)	0.02 (-0.00, 0.05)	6.5% (-0.5, 13.4)	9.4% (7.3, 11.5)	0.03
R5-R20 (kPa/L/s)	-0.02 (-0.05, 0.01)	-12.2% (-31.0, 6.6)	35.4% (15.3, 55.5)	0.03
X5 (kPa/L/s)	-0.01 (-0.05, 0.03)	-4.5% (-18.6, 9.7)	17.1% (8.5, 25.6)	0.04
AX (kPa/L)	-0.02 (-0.51, 0.48)	-0.7% (-18.4, 17.1)	26.9% (14.5, 39.3)	0.50
F_{res} (Hz)	-0.21 (-2.84, 2.42)	-0.9% (-11.7, 10.0)	11.7% (5.3, 18.2)	2.62
PBE (cells/µl)	-19 (-115, 77)	-4.2% (-25.1, 16.8)	21.2% (13.1, 29.2)	96
FeNO (ppb)	-7 (-15, 1)	-12.0% (-25.8, 1.8)	21.0% (14.3, 27.7)	8

*$p<0.05$ **$p<0.01$ ***$p<0.001$ ****$p<0.0001$

In total there were 132 mannitol bronchial challenges performed during the trial. The geometric mean (95%CI) baseline PD_{10} was 67 (34,135). Significant changes in mannitol PD_{10} as the geometric mean fold difference occurred after 8 weeks (Table 26). After week 12 at primary end point, a mean 2.1 (95%CI 1.0, 3.3) doubling difference (DD) in PD_{10} (Bonferroni $p<0.01$) and 2.0 (95%CI 0.9, 3.1) DD in RDR (Bonferroni $p<0.01$) were observed (Figure 17). 12/21 patients experienced a ≥1.0 DD in PD_{10} and RDR (Figure 17) at week 12. 5 patients subsequently became non-responsive to mannitol at 12 weeks.

Table 26 Mean values for mannitol PD_{10}, RDR, spirometry, oscillometry, ACQ, mini-AQLQ and type 2 biomarkers at weeks 0, 2, 4, 8, 12 and 24. Values are shown at 2 and 4 weeks (wk 2, 4) after the first dose, 4 weeks after the 2nd (wk 8) and 3rd doses (wk 12) and 16 weeks after the 3rd dose (wk 24).

	Baseline (Post run-in)	Week 2	Week 4	Week 8	Week 12 (Post Benra)	Geo mean fold diff (CI) at week 12	Week 24
Mannitol PD_{10} (mg)	67	142	157	185*	266**	4.0 (1.9,8.3)	169*
Mannitol RDR (%/mg)	0.1418	0.0679	0.0630	0.0490*	0.0345**	0.2 (0.1,0.5)	0.0435*
						Arith mean diff (CI) at week 12	
FEV_1 (L)	2.37	2.56	2.50	2.48	2.49	0.12 (-0.15, 0.39)	2.58
FEF_{25-75} (L/s)	1.48	1.58	1.56	1.51	1.53	0.05 (-0.27, 0.37)	1.59
FVC (L)	3.62	3.83	3.78	3.79	3.78	0.16 (-0.05, 0.37)	3.89*
FEV_1/FVC	65.5	66.6	66.4	65.8	66.5	1.0 (-1.9, 3.9)	66.8
R5-R20 (kPa/L/s)	0.14	0.14	0.15	0.14	0.14	0.00 (-0.04, 0.04)	0.13
X5 (kPa/L/s)	-0.28	-0.24	-0.22	-0.25	-0.24	0.04 (-0.04, 0.12)	-0.23
AX (kPa/L)	2.77	2.42	2.34	2.49	2.30	-0.46 (-1.43, 0.50)	2.14
PBE (cells/µl)	439	6****			13****	-426 (-574, -277)	33***
EDN (ng/ml)	65.6	28.5****			15.3****	-50.3 (-62.2, -38.5)	19.9****
FeNO (ppb)	51	53	50	65	59	8 (-11, 28)	51
ACQ-6	2.6	1.8*	1.5***	1.3****	1.1****	-1.5 (-2.0, -1.1)	1.5** †
Mini-AQLQ	3.6	4.6***	5.0***	5.2****	5.3****	1.7 (1.1, 2.3)	4.8*

*Bonferroni corrected p values vs baseline: *p<0.05 **p<0.01 ***p<0.001 ****p<0.0001*

P value vs week 12: †p<0.05

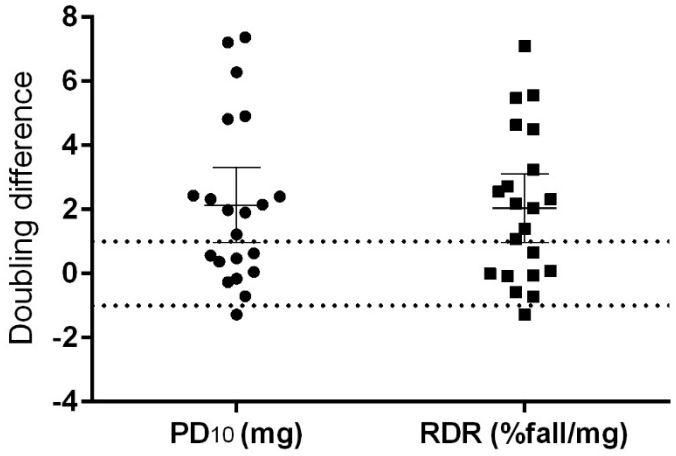

Figure 16 *Individual doubling differences for PD10 and RDR after 12 weeks of benralizumab therapy as change from baseline. Mean and 95% CI also superimposed. Dotted lines depict the within subject biological variability of +/- 1 doubling difference.*

Significant improvements in ACQ-6 and mini-AQLQ scores were demonstrated by week 2 and were sustained over 12 weeks (Table 26). Notably, responder analysis showed 18/21 and 17/21 participants experienced a ≥0.5-unit improvement in ACQ and mini-AQLQ respectively at week 12 exceeding the MCID. After 12 weeks 5 patients had an ACQ score <0.75 indicating good control whilst 13 patients had intermediate control as evidenced by an ACQ score ≥0.75-1.5. All of the 12 patients who responded to mannitol at 12 weeks also had improvements in ACQ and AQLQ exceeding the MCID. For the 9 patients who did not respond to mannitol at 12 weeks (figure 16) represented as a change in PD_{10} within the biological variability of +/- 1.0DD, n=9 and n=6 still experienced a ≥0.5-unit improvement in their ACQ and mini-AQLQ scores respectively. The mean improvements in ACQ and mini-AQLQ scores amounted to 1.6 and 1.7 for mannitol non-responders which were comparable to respective improvements of 1.4 and 1.7 for mannitol responders.

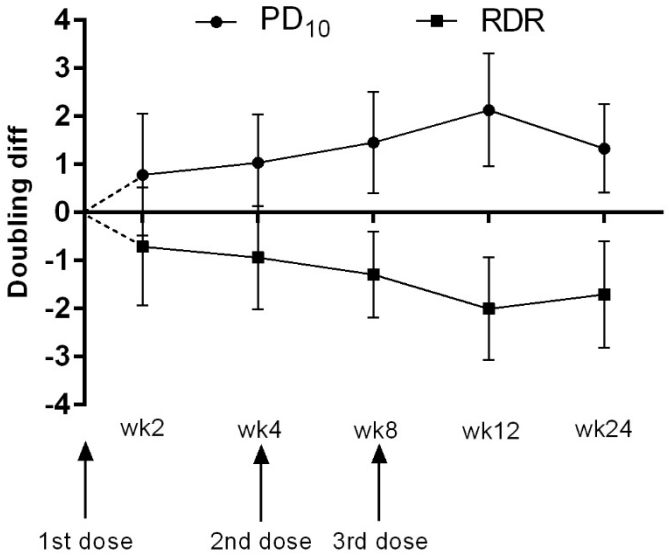

Figure 17 *Mean doubling differences (95%CI) for PD10 and RDR at serial timepoints after benralizumab therapy.*

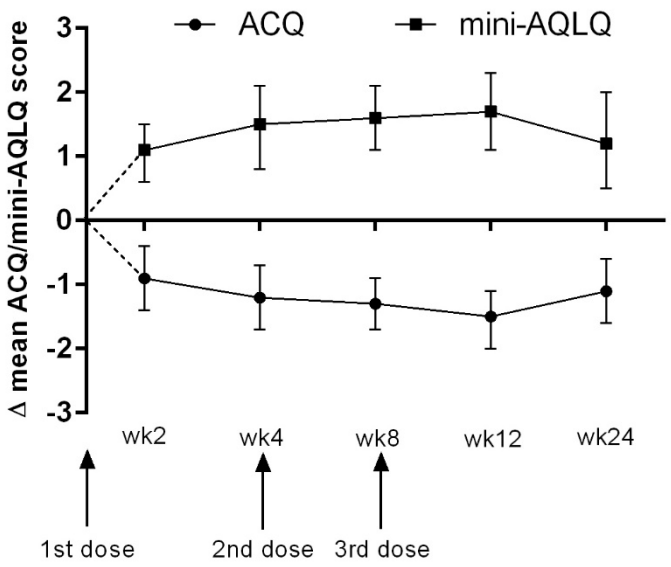

Figure 18 *Mean improvements (95%CI) in ACQ and mini-AQLQ scores at various timepoints after benralizumab therapy. Measures at wk 12 and 24 were performed 4 and 16 weeks following the final 3rd dose.*

Mean individual components of the mini-AQLQ response are shown in Table 27. This demonstrated significant improvements in all domains after 2 weeks which were sustained over 12 weeks except for activity. Table 28 presents a comparison in baseline demographics, asthma control, type 2 biomarkers and FEV_1 in patients whose mannitol PD_{10} response exceeded 1.0 doubling difference after 12 weeks of benralizumab versus those who did not.

Table 27 *Mean individual components of the mini-AQLQ response to benralizumab*

	Baseline	Week 2	Week 4	Week 8	Week 12	Mean difference (95%CI) at week 12	Week 24
Overall	3.6	4.6***	5.0***	5.2****	5.3****	1.7 (1.1, 2.3)	4.8*
Symptoms	3.4	4.6****	5.2****	5.4****	5.5****	2.0 (1.4, 2.7)	5.0**
Environment	3.4	4.0	4.5*	4.3*	4.7**	1.3 (0.7, 2.0)	4.4
Emotions	3.3	4.2**	4.9**	5.1***	5.1**	1.8 (0.9, 2.7)	4.7
Activity	4.1	5.1*	5.3**	5.6***	4.9	0.8 (0.0, 1.6)	5.1

*Bonferroni corrected comparisons vs baseline *p<0.05 **p<0.01 ***p<0.001 ****p<0.0001*

Table 28 Comparison of baseline demographics, asthma control, type 2 biomarkers and FEV_1 in patients whose mannitol PD_{10} response exceeded 1.0 doubling doses after 3 doses of benralizumab versus those who did not.

	ΔPD_{10} ≥1.0 DD (n=12)	**ΔPD_{10} <1.0 DD (n=9)**
ICS BDP (µg)	1933 (67)	1889 (111)
Age	52 (4)	54 (7)
BMI (kg/m^2)	29.3 (1.6)	31.1 (1.9)
ACQ	2.5 (0.2)	2.8 (0.4)
PBE (cells/µl)	409 (95)	478 (104)
FeNO (ppb)	37 (6)	68 (19)
EDN (ng/ml)	55.1 (5.7)	79.6 (9.8) *
FEV_1 (%)	74.7 (5.1)	87.6 (6.3)

*p<0.05 mean (SEM)

No significant changes in spirometry or oscillometry were observed after 12 weeks (Table 26). Peripheral blood eosinophils were significantly depleted by week 2 and sustained over 12 weeks, whereas numerical increases in FeNO over 12 weeks were not significant (Table 26). Serum EDN levels significantly fell from baseline to weeks 2 (57% fall) and 12 (77% fall) (figure 19).

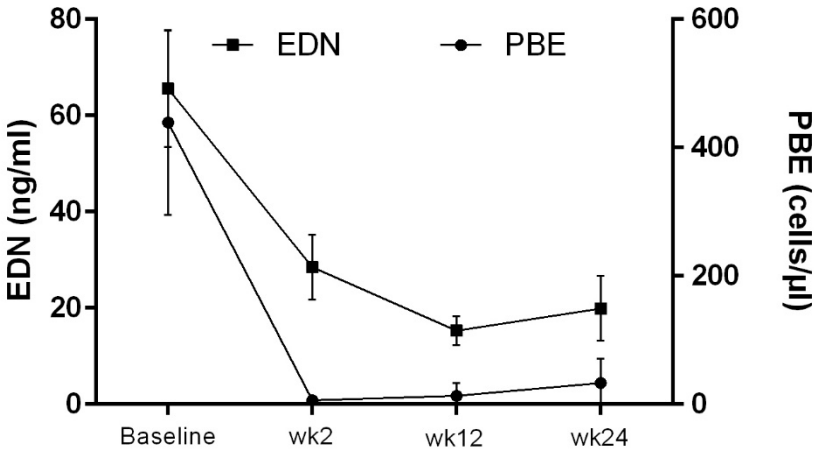

Figure 19 *Suppression of peripheral blood eosinophils and eosinophil derived neurotoxin post benralizumab*

PEF, symptoms and reliever salbutamol use from patient diary cards all significantly improved after three doses of benralizumab i.e., week 12 versus baseline. (Table 29). Notably, the lower CI for the change in PEF at week 12 compared to baseline exceeded the MCID of 19L/min (Figure 20).

Table 29 Mean peak expiratory flow rate, patient subjective symptoms and reliever use in 7 days prior to respective visit

	Baseline	Week 2	Week 4	Week 8	Week 12	Mean difference (95%CI) at week 12	Week 24
PEF (L/min)	357	388*	400**	398**	404**	48 (21, 74)	404*
Symptoms	1.7	1.3*	1.3**	1.1***	1.0****	-0.7 (-0.9, -0.5)	0.9***
Reliever use	3	2*	2*	1**	1*	-2 (-3, -0)	2*

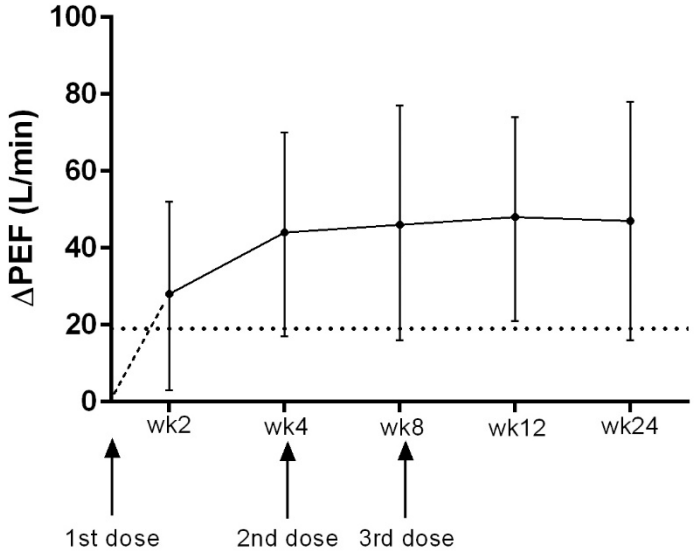

Figure 20 *Absolute change in peak expiratory flow compared to baseline as means and 95%CI. Interrupted line represents minimal clinically important difference for PEF of 19L/min. Measures at wk 12 and 24 were performed 4 and 16 weeks following the final 3rd dose.*

After the 12-week washout period there was a numerical non-significant trend towards worsening of mannitol PD_{10} when comparing values between 4 months and 1 month after the last dose of benralizumab, i.e., week 24 versus week 12, amounting to a mean -0.7 DD (95%CI -1.6, 0.3) difference. For the same time point comparison, a significant worsening in ACQ and a worsening in mini-AQLQ equalling MCID was observed (table 26), despite no differences in peripheral blood eosinophils. However, values for ACQ and mini-AQLQ remained significantly better when comparing week 24 versus baseline. No differences in spirometry or oscillometry measurements were detected between weeks 12 and 24, while changes in PEF persisted 4 months after the final dose was given at week 24.

DISCUSSION

In this pragmatic study we have demonstrated that eosinophil depletion due to benralizumab was associated with clinically relevant attenuation of indirect AHR at end point after 12 weeks in patients with uncontrolled SEA. This was accompanied by significant improvements in asthma control and quality of life. Notably at 12 weeks, the lower 95%CI for DD shift in mannitol PD_{10} was 1.0 as compared to biological variability of +/-1.0 DD. As a reference, the within subject biological variabilities in the placebo arm for mannitol AHR in CASCADE[129] and

UPSTREAM[323] were 0.57 and 1.0 DD respectively. Therefore, we opted to use 1.0 DD to represent the minimal change that must be exceeded for a clinically relevant treatment effect.

One interesting finding from this study was the variability in response defined by PD_{10} DD at 12 weeks (figure 16) despite all patients exhibiting severe eosinophilic asthma. It is perhaps worth mentioning here that 5 patients experienced improvements in PD_{10} that exceeded 4.0 DD at 12 weeks. The previous definition of a biologic super-responder[114] was subjectively based on AER reduction and improvements in FEV_1 and asthma control. As such this study may have identified AHR as an important aspect of the biologic super-responder definition that is still undergoing development.[340]

The lower 95% CIs for ACQ exceeded the MCID of 0.5 at weeks 4, 8 and 12. Indeed by 12 weeks the lower 95%CI exceeded twice the MCID at >1.0. These findings are especially clinically relevant as it has previously been determined that each 1.0 unit increase in ACQ is approximately associated with a 50% increased risk of asthma exacerbation.[242] For mini-AQLQ scores the lower 95% CIs exceeded 0.5 at 2, 4, 8 and 12 weeks (figure 18).

We observed significantly improved PEF at week 12 versus baseline with the lower CI for the change exceeding the MCID of 19L/min.[64] One likely explanation for the apparent disconnect between improvements in PEF but not FEV_1 is that serial values for PEF were averaged during the seven days prior to the particular study visit. Thus, serial measures of peak flow are always more likely to detect subtle changes in airway calibre as opposed to spot laboratory measures using spirometry.

The putative mechanism for benralizumab on indirect mannitol challenge is likely to be mediated via intraepithelial eosinophils in attenuating endogenous AHR in asthma, at least in part by leukotriene D4 release from depleted eosinophils.[341,342] Mast cells also play a key role in indirect AHR via IL-33 signalling,[343] with early evidence suggesting that eosinophils may regulate mast cell function.[341] Previous studies with mepolizumab reported no effect on direct acting challenge to either methacholine or histamine inferring that blocking IL-5 has no effect on airway smooth muscle.[317,344] This discrepancy from our study could potentially be explained by three reasons. First, benralizumab but not mepolizumab significantly reduces airway eosinophilia.[345,346] Second, indirect airway challenge is more closely associated with

bronchial inflammation, which in turn is suppressed with biologic therapy, compared to direct airway challenge.[319] Third, the study by Leckie et al only used a single dose of mepolizumab raising the question of whether the duration of therapy was adequately long enough to detect a treatment response. The study by Haldar et al did not evaluate AHR as the primary outcome and therefore may have been underpowered to answer this question.

EDN is an eosinophil degranulation protein that better reflects asthma control compared to blood eosinophils.[347] Furthermore, EDN is associated with acute asthma exacerbations and airway hyperresponsiveness.[348] In this study we have demonstrated that whilst PBE counts neared complete depletion by week 2 post benralizumab, while EDN levels progressively drifted down from baseline to week 12 where there was 77% suppression (table 26 and figure 19). This incomplete suppression of EDN could potentially be explained by a persistent reservoir of airway eosinophils that are not entirely depleted from benralizumab therapy.[318] Alternatively, although the greatest supply of EDN is derived from eosinophils, it is recognised that other sources of EDN exist including macrophages, monocytes and neutrophils.[349]

We appreciate there are potential limitations associated with our study. Firstly, it was not placebo controlled. Accepting a putative 1.0 DD change in AHR with placebo we do not feel the 2.1 DD change in AHR with benralizumab represents regression to the mean over 12 weeks compared to baseline, especially when using rigorous Bonferroni corrected p values. It is also perhaps worth mentioning that the 5/21 patients who were no longer mannitol responsive at week 12 (i.e., PD_{10} ≥635mg) had their PD_{10} values censored at the maximum dose (635mg) for statistical analysis, therefore likely underestimating the true effect of benralizumab in attenuating AHR in such patients.

Procedures for this study were particularly difficult to execute as they coincided with the peak of the COVID-19 pandemic associated with stringent protective measures involving aerosol generating procedures especially early on in the pandemic. We hope we might have mitigated the lack of a placebo controlled arm by calculating biological variability values during the run-in period[5] that can be used as surrogates for the minimal change that must occur for a clinically significant treatment effect.

Furthermore, we acknowledge that it is increasingly difficult to justify randomising severe uncontrolled asthma patients, who were initially shielding, onto placebo when there is

abundant evidence for the efficacy of benralizumab. This would not be the case for evaluation of a novel biologic which patients could not access on the NHS. Our particular strategy was to inform patients prior to enrolment that they would be referred onto the severe asthma multidisciplinary team at the conclusion of the trial if there was evidence of a good response to benralizumab.

We included a washout period to assess if the effects of benralizumab might have started to wear off after a period of four months of the last dose. Despite no differences in peripheral blood eosinophils when comparing four and one months after the last dose of benralizumab (week 24 vs week 12), our patients experienced a small but significant worsening of both their ACQ and mini-AQLQ scores, while mannitol PD_{10} demonstrated a numerical non-significant worsening. Nonetheless values for week 24 remained significantly better than baseline for mannitol PD_{10}, RDR, ACQ and mini-AQLQ. We duly acknowledge that blood eosinophils may not necessarily reflect lung eosinophils even though persistent depletion in blood was observed at week 24.

We feel improvements in mannitol AHR were unlikely due to increased patient adherence to ICS as one would otherwise expect FeNO values to fall over 12 weeks.[96] Instead, FeNO levels increased non significantly by 8ppb from baseline to week 12. Notably, we did not document ICS/LABA adherence in the present study. Lastly, although the sub-analysis in table 28 is likely underpowered, we noted a significantly higher baseline EDN level in patients who had an improvement in mannitol PD_{10} <1.0 doubling difference at 12 weeks, possibly warranting further investigation in future studies.

In conclusion, we have shown that eosinophil depletion with benralizumab attenuates indirect mannitol airway hyperresponsiveness, while also improving domiciliary peak flow, asthma control and quality of life in patients with severe uncontrolled eosinophilic asthma.

Conclusion of thesis

I am grateful once again to have been given the opportunity to embark on this academic journey and cannot help but feel this has been the most productive three years of my career so far. In this thesis I have summarised the literature base surrounding biologics including its effect on the small airways and airway hyperresponsiveness in persistent asthma. Through six initial studies I contributed additional or strengthened existing knowledge around relevant topics including small airways dysfunction, nasal polyposis, mucus plugging and bronchial wall thickness. In the final chapter I have demonstrated that eosinophil depletion using anti-IL5Rα therapy improves airway hyperresponsiveness in patients with severe asthma.

This thesis has undoubtedly left me asking more questions which I hope to get involved with answering in the future. For instance, do all biologics improve airway hyperresponsiveness? In this regard, I eagerly await the results of the clinical trials presently investigating the effect of anti-IL4Rα therapy on airway hyperresponsiveness in severe asthma using mannitol (EudraCT number 2021-005593-25) and methacholine (NCT03884842). One randomised controlled trial (NCT04400318) looking at the effect of dupilumab on mucus plugging and bronchial wall thickness is also of significant interest. Lastly, perhaps one of the biggest questions in this exciting area is whether one biologic is superior to another but at present we are still awaiting head-to-head trials.

Publications arising from this thesis

1. **Chan R**, Misirovs R, Lipworth B. Repeatability of impulse oscillometry in patients with severe asthma. Eur Respir J. 2021 Dec 31;59(1):2101679. doi: 10.1183/13993003.01679-2021. PMID: 34625483.

2. **Chan R**, RuiWen Kuo C, Lipworth B. Pragmatic Clinical Perspective on Biologics for Severe Refractory Type 2 Asthma. J Allergy Clin Immunol Pract. 2020 Nov-Dec;8(10):3363-3370. doi: 10.1016/j.jaip.2020.06.048. Epub 2020 Jul 13. PMID: 32673880.

3. **Chan R**, Lipworth B. Impact of nasal polyps on endotype and phenotype in patients with moderate to severe asthma. Ann Allergy Asthma Immunol. 2021 Nov;127(5):548-552. doi: 10.1016/j.anai.2021.06.007. Epub 2021 Jun 18. PMID: 34153444.

4. **Chan R**, Stewart K, Misirovs R, Lipworth B. Targeting downstream type 2 cytokines or upstream epithelial alarmins for severe asthma. J Allergy Clin Immunol Pract. 2022 Feb 4:S2213-2198(22)00120-9. doi: 10.1016/j.jaip.2022.01.040. Epub ahead of print. PMID: 35131510.

5. **Chan R**, Lipworth BJ. Oscillometry bronchodilator response in adult moderate to severe eosinophilic asthma patients: A prospective cohort study. Clin Exp Allergy. 2022 Jun 16. doi: 10.1111/cea.14185. Epub ahead of print. PMID: 35707948.

6. **Chan R**, Lipworth B. Interactions between spirometry and oscillometry in patients with moderate to severe asthma. Eur Respir J. 2022 Aug 18:2200543. doi: 10.1183/13993003.00543-2022. Epub ahead of print. PMID: 35981746.

7. **Chan R**, Duraikannu C, Lipworth B. Clinical associations of mucus plugging in moderate to severe asthma. J Allergy Clin Immunol Pract. 2022 Sep 21:S2213-2198(22)00943-6. doi: 10.1016/j.jaip.2022.09.008. Epub ahead of print. PMID: 36152990.

8. **Chan R**, Lipworth BJ. Impact of Biologic Therapy on the Small Airways Asthma Phenotype. Lung. 2022 Oct 14. doi: 10.1007/s00408-022-00579-2. Epub ahead of print. PMID: 36239786.

9. **Chan R**, Kuo CR, Jabbal S, Lipworth B. Eosinophil depletion with benralizumab is associated with attenuated mannitol airway hyperresponsiveness in severe uncontrolled eosinophilic asthma. Journal of Allergy and Clinical Immunology.

10. **Chan R**, Duraikannu C, Thouseef MJ, Lipworth B. Impaired Respiratory System Resistance and Reactance Are Associated With Bronchial Wall Thickening in Persistent Asthma. J Allergy Clin Immunol Pract. 2023 Jan 11:S2213-2198(23)00051-X. doi: 10.1016/j.jaip.2022.12.040. Epub ahead of print. PMID: 36639055.

Presentations arising from this thesis

British Thoracic Society

- Bronchodilator response for airway oscillometry in patients with severe eosinophilic asthma. Thorax 2021;76(Suppl 2): A31 – 32 (Spoken)
- Repeatability of impulse oscillometry in patients with severe asthma. Thorax 2021;76(Suppl 2): A32 (Spoken)

American Thoracic Society

- Benralizumab improves mannitol airway hyperresponsiveness in uncontrolled severe eosinophilic asthma. Am J Respir Crit Care Med 2022;205:A5698 (Poster)

European Respiratory Society

- Bronchodilator response for oscillometry in severe eosinophilic asthma. ERS, Barcelona 2022 (Poster)
- Interactions between spirometry and oscillometry in patients with moderate to severe asthma. ERS, Barcelona 2022 (Poster)
- Airway hyperresponsiveness in uncontrolled severe eosinophilic asthma with anti-IL5rα. ERS, Barcelona 2022 (Poster)
- Clinical predictors and phenotypic associations of mucus plugging in moderate to severe asthma. ERS, Barcelona 2022 (Spoken)

References

1. Chan R, RuiWen Kuo C, Lipworth B. Pragmatic Clinical Perspective on Biologics for Severe Refractory Type 2 Asthma. *J Allergy Clin Immunol Pract* 2020.
2. Chan R, Stewart K, Misirovs R, Lipworth B. Targeting downstream type 2 cytokines or upstream epithelial alarmins for severe asthma. *J Allergy Clin Immunol Pract* 2022; **doi: 10.1016/j.jaip.2022.01.040.**
3. Chan R, Lipworth B. Impact of nasal polyps on endotype and phenotype in patients with moderate to severe asthma. *Ann Allergy Asthma Immunol* 2021.
4. Chan R, Lipworth BJ. Impact of Biologic Therapy on the Small Airways Asthma Phenotype. *Lung* 2022.
5. Chan R, Misirovs R, Lipworth B. Repeatability of impulse oscillometry in patients with severe asthma. *European Respiratory Journal* 2021: 2101679.
6. Chan R, Lipworth B. Interactions between spirometry and oscillometry in patients with moderate to severe asthma. *Eur Respir J* 2022.
7. Chan R, Lipworth BJ. Oscillometry bronchodilator response in adult moderate to severe eosinophilic asthma patients: A prospective cohort study. *Clin Exp Allergy* 2022.
8. Chan R, Duraikannu C, Lipworth B. Clinical associations of mucus plugging in moderate to severe asthma. *J Allergy Clin Immunol Pract* 2022.
9. Chan R, Duraikannu C, Thouseef MJ, Lipworth B. Impaired Respiratory System Resistance and Reactance Are Associated With Bronchial Wall Thickening in Persistent Asthma. *J Allergy Clin Immunol Pract* 2023.
10. Chan R, Kuo CR, Jabbal S, Lipworth B. Eosinophil depletion with benralizumab is associated with attenuated mannitol airway hyperresponsiveness in severe uncontrolled eosinophilic asthma. *Journal of Allergy and Clinical Immunology*.
11. Agache I, Akdis CA, Akdis M, et al. EAACI Biologicals Guidelines-Recommendations for severe asthma. *Allergy* 2021; **76**(1): 14-44.
12. McGregor MC, Krings JG, Nair P, Castro M. Role of Biologics in Asthma. *Am J Respir Crit Care Med* 2019; **199**(4): 433-45.
13. Roth M, Stolz D. Biomarkers and personalised medicine for asthma. *European Respiratory Journal* 2019; **53**(1): 1802094.
14. Garon EB, Rizvi NA, Hui R, et al. Pembrolizumab for the Treatment of Non–Small-Cell Lung Cancer. *New England Journal of Medicine* 2015; **372**(21): 2018-28.
15. McDonald VM, Clark VL, Cordova-Rivera L, Wark PAB, Baines KJ, Gibson PG. Targeting treatable traits in severe asthma: a randomised controlled trial. *European Respiratory Journal* 2020; **55**(3): 1901509.
16. Chung KF, Adcock IM. Precision medicine for the discovery of treatable mechanisms in severe asthma. *Allergy* 2019; **74**(9): 1649-59.
17. Lefaudeux D, De Meulder B, Loza MJ, et al. U-BIOPRED clinical adult asthma clusters linked to a subset of sputum omics. *J Allergy Clin Immunol* 2017; **139**(6): 1797-807.
18. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention Main Report 2021. www.ginasthma.org. Accessed March 21, 2022.
19. Global Initiative for Asthma. Difficult-to-treat & severe asthma in adolescent and adult patients. Diagnosis and management. https://ginasthma.org/wp-content/uploads/2019/04/GINA-Severe-asthma-Pocket-Guide-v2.0-wms-1.pdf Accessed September 06, 2021.

20. Busse W, Corren J, Lanier BQ, et al. Omalizumab, anti-IgE recombinant humanized monoclonal antibody, for the treatment of severe allergic asthma. *J Allergy Clin Immunol* 2001; **108**(2): 184-90.
21. Barnes PJ. Intrinsic asthma: not so different from allergic asthma but driven by superantigens? *Clin Exp Allergy* 2009; **39**(8): 1145-51.
22. Humbert M, Durham SR, Ying S, et al. IL-4 and IL-5 mRNA and protein in bronchial biopsies from patients with atopic and nonatopic asthma: evidence against "intrinsic" asthma being a distinct immunopathologic entity. *Am J Respir Crit Care Med* 1996; **154**(5): 1497-504.
23. Humbert M, Menz G, Ying S, et al. The immunopathology of extrinsic (atopic) and intrinsic (non-atopic) asthma: more similarities than differences. *Immunol Today* 1999; **20**(11): 528-33.
24. Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. *Respirology* 2006; **11**(1): 54-61.
25. Green RH, Brightling CE, Woltmann G, Parker D, Wardlaw AJ, Pavord ID. Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids. *Thorax* 2002; **57**(10): 875-9.
26. Fahy JV. Type 2 inflammation in asthma--present in most, absent in many. *Nat Rev Immunol* 2015; **15**(1): 57-65.
27. Spellberg B, Edwards JE, Jr. Type 1/Type 2 immunity in infectious diseases. *Clin Infect Dis* 2001; **32**(1): 76-102.
28. Voehringer D, Reese TA, Huang X, Shinkai K, Locksley RM. Type 2 immunity is controlled by IL-4/IL-13 expression in hematopoietic non-eosinophil cells of the innate immune system. *J Exp Med* 2006; **203**(6): 1435-46.
29. Kavanagh JE, Hearn AP, Jackson DJ. A pragmatic guide to choosing biologic therapies in severe asthma. *Breathe (Sheff)* 2021; **17**(4): 210144.
30. Chan R, RuiWen Kuo C, Lipworth B. Pragmatic Clinical Perspective on Biologics for Severe Refractory Type 2 Asthma. *J Allergy Clin Immunol Pract* 2020; **8**(10): 3363-70.
31. Mitchell PD, O'Byrne PM. Epithelial-Derived Cytokines in Asthma. *Chest* 2017; **151**(6): 1338-44.
32. Porsbjerg CM, Sverrild A, Lloyd CM, Menzies-Gow AN, Bel EH. Anti-alarmins in asthma: targeting the airway epithelium with next-generation biologics. *Eur Respir J* 2020; **56**(5).
33. Althoff MD, Ghincea A, Wood LG, Holguin F, Sharma S. Asthma and Three Colinear Comorbidities: Obesity, OSA, and GERD. *J Allergy Clin Immunol Pract* 2021.
34. Lual KP, Yizengaw MA. Factors Associated with Suboptimal Control of Asthma among Adult Asthma Patients: A Cross-sectional Study. *Open Respir Med J* 2021; **15**: 35-42.
35. SIGN 158 British guideline on the management of asthma. Date accessed September 13, 2021.
36. Lipworth B, Chan R, Kuo CR. Anti-inflammatory reliever therapy for asthma. *Annals of Allergy, Asthma & Immunology* 2020; **124**(1): 13-5.
37. Levy ML, Winter R. Asthma deaths: what now? *Thorax* 2015; **70**(3): 209-10.
38. Beasley R, Holliday M, Reddel HK, et al. Controlled Trial of Budesonide–Formoterol as Needed for Mild Asthma. *New England Journal of Medicine* 2019; **380**(21): 2020-30.
39. Hardy J, Baggott C, Fingleton J, et al. Budesonide-formoterol reliever therapy versus maintenance budesonide plus terbutaline reliever therapy in adults with mild to moderate asthma (PRACTICAL): a 52-week, open-label, multicentre, superiority, randomised controlled trial. *Lancet* 2019.

40. O'Byrne PM, FitzGerald JM, Bateman ED, et al. Inhaled Combined Budesonide–Formoterol as Needed in Mild Asthma. *New England Journal of Medicine* 2018; **378**(20): 1865-76.
41. Bateman ED, Reddel HK, O'Byrne PM, et al. As-Needed Budesonide–Formoterol versus Maintenance Budesonide in Mild Asthma. *New England Journal of Medicine* 2018; **378**(20): 1877-87.
42. Denlinger LC, Heymann P, Lutter R, Gern JE. Exacerbation-Prone Asthma. *J Allergy Clin Immunol Pract* 2020; **8**(2): 474-82.
43. Peters MC, Kerr S, Dunican EM, et al. Refractory airway type 2 inflammation in a large subgroup of asthmatic patients treated with inhaled corticosteroids. *J Allergy Clin Immunol* 2019; **143**(1): 104-13.e14.
44. Coverstone AM, Seibold MA, Peters MC. Diagnosis and Management of T2-High Asthma. *J Allergy Clin Immunol Pract* 2020; **8**(2): 442-50.
45. Fitzpatrick AM, Chipps BE, Holguin F, Woodruff PG. T2-"Low" Asthma: Overview and Management Strategies. *J Allergy Clin Immunol Pract* 2020; **8**(2): 452-63.
46. Akar-Ghibril N, Casale T, Custovic A, Phipatanakul W. Allergic Endotypes and Phenotypes of Asthma. *J Allergy Clin Immunol Pract* 2020; **8**(2): 429-40.
47. Nelson RK, Bush A, Stokes J, Nair P, Akuthota P. Eosinophilic Asthma. *J Allergy Clin Immunol Pract* 2020; **8**(2): 465-73.
48. Martin RJ, Bel EH, Pavord ID, Price D, Reddel HK. Defining severe obstructive lung disease in the biologic era: an endotype-based approach. *Eur Respir J* 2019; **54**(5).
49. Lipworth BJ, Jabbal S. Un-diagnosing persistent adult asthma. *European Respiratory Journal* 2017; **50**(5): 1701433.
50. Hew M, Menzies-Gow A, Hull JH, et al. Systematic Assessment of Difficult-to-Treat Asthma: Principles and Perspectives. *The Journal of Allergy and Clinical Immunology: In Practice* 2020.
51. Solèr M, Matz J, Townley R, et al. The anti-IgE antibody omalizumab reduces exacerbations and steroid requirement in allergic asthmatics. *European Respiratory Journal* 2001; **18**(2): 254-61.
52. Castro M, Corren J, Pavord ID, et al. Dupilumab Efficacy and Safety in Moderate-to-Severe Uncontrolled Asthma. *N Engl J Med* 2018; **378**(26): 2486-96.
53. Pavord ID, Korn S, Howarth P, et al. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. *Lancet* 2012; **380**(9842): 651-9.
54. Busse WW, Maspero JF, Rabe KF, et al. Liberty Asthma QUEST: Phase 3 Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Study to Evaluate Dupilumab Efficacy/Safety in Patients with Uncontrolled, Moderate-to-Severe Asthma. *Adv Ther* 2018; **35**(5): 737-48.
55. Nagasaki T, Sato K, Kume N, et al. The prevalence and disease burden of severe eosinophilic asthma in Japan. *Journal of Asthma* 2019; **56**(11): 1147-58.
56. Heaney LG, Perez de Llano L, Al-Ahmad M, et al. Eosinophilic and Noneosinophilic Asthma: An Expert Consensus Framework to Characterize Phenotypes in a Global Real-Life Severe Asthma Cohort. *Chest* 2021; **160**(3): 814-30.
57. Azim A, Newell C, Barber C, et al. Clinical evaluation of type 2 disease status in a real-world population of difficult to manage asthma using historic electronic healthcare records of blood eosinophil counts. *Clinical & Experimental Allergy* 2021; **51**(6): 811-20.

58. Arbes SJ, Jr., Gergen PJ, Vaughn B, Zeldin DC. Asthma cases attributable to atopy: results from the Third National Health and Nutrition Examination Survey. *The Journal of allergy and clinical immunology* 2007; **120**(5): 1139-45.
59. Wenzel SE, Busse WW. Severe asthma: lessons from the Severe Asthma Research Program. *J Allergy Clin Immunol* 2007; **119**(1): 14-21; quiz 2-3.
60. Corren J, Castro M, O'Riordan T, et al. Dupilumab Efficacy in Patients with Uncontrolled, Moderate-to-Severe Allergic Asthma. *J Allergy Clin Immunol Pract* 2019.
61. Lipworth B, Chan R, Kuo C. Allergic burden and response to dupilumab. *J Allergy Clin Immunol Pract* 2020; **8**(2): 822.
62. Rakowski E, Zhao S, Liu M, et al. Variability of blood eosinophils in patients in a clinic for severe asthma. *Clin Exp Allergy* 2019; **49**(2): 163-70.
63. Farne HA, Wilson A, Powell C, Bax L, Milan SJ. Anti-IL5 therapies for asthma. *Cochrane Database of Systematic Reviews* 2017; (9).
64. Santanello NC, Zhang J, Seidenberg B, Reiss TF, Barber BL. What are minimal important changes for asthma measures in a clinical trial? *Eur Respir J* 1999; **14**(1): 23-7.
65. Juniper EF, Svensson K, Mork AC, Stahl E. Measurement properties and interpretation of three shortened versions of the asthma control questionnaire. *Respir Med* 2005; **99**(5): 553-8.
66. Kouro T, Takatsu K. IL-5- and eosinophil-mediated inflammation: from discovery to therapy. *Int Immunol* 2009; **21**(12): 1303-9.
67. Ortega HG, Yancey SW, Mayer B, et al. Severe eosinophilic asthma treated with mepolizumab stratified by baseline eosinophil thresholds: a secondary analysis of the DREAM and MENSA studies. *Lancet Respir Med* 2016; **4**(7): 549-56.
68. Corren J, Weinstein S, Janka L, Zangrilli J, Garin M. Phase 3 Study of Reslizumab in Patients With Poorly Controlled Asthma: Effects Across a Broad Range of Eosinophil Counts. *Chest* 2016; **150**(4): 799-810.
69. Busse WW, Bleecker ER, FitzGerald JM, et al. Long-term safety and efficacy of benralizumab in patients with severe, uncontrolled asthma: 1-year results from the BORA phase 3 extension trial. *Lancet Respir Med* 2019; **7**(1): 46-59.
70. Harvey ES, Langton D, Katelaris C, et al. Mepolizumab effectiveness and identification of super-responders in severe asthma. *European Respiratory Journal* 2020: 1902420.
71. Normansell R, Walker S, Milan SJ, Walters EH, Nair P. Omalizumab for asthma in adults and children. *Cochrane Database Syst Rev* 2014; (1): Cd003559.
72. Chipps BE, Newbold P, Hirsch I, Trudo F, Goldman M. Benralizumab efficacy by atopy status and serum immunoglobulin E for patients with severe, uncontrolled asthma. *Ann Allergy Asthma Immunol* 2018; **120**(5): 504-11.e4.
73. Shrimanker R, Keene O, Hynes G, Wenzel S, Yancey S, Pavord ID. Prognostic and Predictive Value of Blood Eosinophil Count, Fractional Exhaled Nitric Oxide, and Their Combination in Severe Asthma: A Post Hoc Analysis. *Am J Respir Crit Care Med* 2019; **200**(10): 1308-12.
74. Couillard S, Laugerud A, Jabeen M, et al. Derivation of a prototype asthma attack risk scale centred on blood eosinophils and exhaled nitric oxide. *Thorax* 2022; **77**(2): 199-202.
75. Russell RJ, Chachi L, FitzGerald JM, et al. Effect of tralokinumab, an interleukin-13 neutralising monoclonal antibody, on eosinophilic airway inflammation in uncontrolled moderate-to-severe asthma (MESOS): a multicentre, double-blind, randomised, placebo-controlled phase 2 trial. *Lancet Respir Med* 2018; **6**(7): 499-510.

76. Mukherjee M, Forero DF, Tran S, et al. Sub-optimal treatment response to anti-IL-5 monoclonal antibodies in severe eosinophilic asthmatics with airway autoimmune phenomena. *Eur Respir J* 2020.
77. Panettieri RA, Jr., Sjobring U, Peterffy A, et al. Tralokinumab for severe, uncontrolled asthma (STRATOS 1 and STRATOS 2): two randomised, double-blind, placebo-controlled, phase 3 clinical trials. *Lancet Respir Med* 2018; **6**(7): 511-25.
78. Hanania NA, Korenblat P, Chapman KR, et al. Efficacy and safety of lebrikizumab in patients with uncontrolled asthma (LAVOLTA I and LAVOLTA II): replicate, phase 3, randomised, double-blind, placebo-controlled trials. *Lancet Respir Med* 2016; **4**(10): 781-96.
79. Lipworth B, Jabbal S, Kuo CR. Anti-interleukin 13 for asthma: stick or twist? *Lancet Respir Med* 2018; **6**(9): e46-e7.
80. Charles D, Shanley J, Temple SN, Rattu A, Khaleva E, Roberts G. Real-world efficacy of treatment with benralizumab, dupilumab, mepolizumab and reslizumab for severe asthma: A systematic review and meta-analysis. *Clin Exp Allergy* 2022; **52**(5): 616-27.
81. Hearn AP, Kavanagh J, d'Ancona G, et al. The relationship between Feno and effectiveness of mepolizumab and benralizumab in severe eosinophilic asthma. *The Journal of Allergy and Clinical Immunology: In Practice* 2021; **9**(5): 2093-6.e1.
82. Bateman ED, Khan AH, Xu Y, et al. Pairwise indirect treatment comparison of dupilumab versus other biologics in patients with uncontrolled persistent asthma. *Respiratory Medicine* 2020: 105991.
83. Humbert M, Taille C, Mala L, Le Gros V, Just J, Molimard M. Omalizumab effectiveness in patients with severe allergic asthma according to blood eosinophil count: the STELLAIR study. *Eur Respir J* 2018; **51**(5).
84. Bousquet J, Rabe K, Humbert M, et al. Predicting and evaluating response to omalizumab in patients with severe allergic asthma. *Respir Med* 2007; **101**(7): 1483-92.
85. Hanania NA, Wenzel S, Rosen K, et al. Exploring the effects of omalizumab in allergic asthma: an analysis of biomarkers in the EXTRA study. *Am J Respir Crit Care Med* 2013; **187**(8): 804-11.
86. Stein ML, Collins MH, Villanueva JM, et al. Anti-IL-5 (mepolizumab) therapy for eosinophilic esophagitis. *J Allergy Clin Immunol* 2006; **118**(6): 1312-9.
87. Hirano I, Dellon ES, Hamilton JD, et al. Efficacy of Dupilumab in a Phase 2 Randomized Trial of Adults With Active Eosinophilic Esophagitis. *Gastroenterology* 2020; **158**(1): 111-22.e10.
88. Dellon ES, Rothenberg ME, Collins MH, et al. Dupilumab in Adults and Adolescents with Eosinophilic Esophagitis. *New England Journal of Medicine* 2022; **387**(25): 2317-30.
89. Bleecker ER, Wechsler ME, FitzGerald JM, et al. Baseline patient factors impact on the clinical efficacy of benralizumab for severe asthma. *Eur Respir J* 2018; **52**(4).
90. Chan R, Kuo CR, Lipworth B. Disconnect between effects of mepolizumab on severe eosinophilic asthma and chronic rhinosinusitis with nasal polyps. *J Allergy Clin Immunol Pract* 2020.
91. Bachert C, Han JK, Desrosiers M, et al. Efficacy and safety of dupilumab in patients with severe chronic rhinosinusitis with nasal polyps (LIBERTY NP SINUS-24 and LIBERTY NP SINUS-52): results from two multicentre, randomised, double-blind, placebo-controlled, parallel-group phase 3 trials. *The Lancet* 2019.
92. Wechsler ME, Akuthota P, Jayne D, et al. Mepolizumab or Placebo for Eosinophilic Granulomatosis with Polyangiitis. *N Engl J Med* 2017; **376**(20): 1921-32.

93. Alexis AF, Rendon M, Silverberg JI, et al. Efficacy of Dupilumab in Different Racial Subgroups of Adults With Moderate-to-Severe Atopic Dermatitis in Three Randomized, Placebo-Controlled Phase 3 Trials. *J Drugs Dermatol* 2019; **18**(8): 804-13.
94. Maurer M, Rosén K, Hsieh H-J, et al. Omalizumab for the Treatment of Chronic Idiopathic or Spontaneous Urticaria. *New England Journal of Medicine* 2013; **368**(10): 924-35.
95. Dweik RA, Boggs PB, Erzurum SC, et al. An Official ATS Clinical Practice Guideline: Interpretation of Exhaled Nitric Oxide Levels (FeNO) for Clinical Applications. *American Journal of Respiratory and Critical Care Medicine* 2011; **184**(5): 602-15.
96. Kuo CR, Spears M, Haughney J, et al. Scottish consensus statement on the role of FeNO in adult asthma. *Respir Med* 2019; **155**: 54-7.
97. Maniscalco M, Calabrese C, D'Amato M, et al. Association between exhaled nitric oxide and nasal polyposis in severe asthma. *Respir Med* 2019; **152**: 20-4.
98. Westerhof GA, Coumou H, de Nijs SB, Weersink EJ, Bel EH. Clinical predictors of remission and persistence of adult-onset asthma. *J Allergy Clin Immunol* 2018; **141**(1): 104-9.e3.
99. Kavanagh JE, Hearn AP, Dhariwal J, et al. Real-World Effectiveness of Benralizumab in Severe Eosinophilic Asthma. *Chest* 2021; **159**(2): 496-506.
100. FitzGerald JM, Bleecker ER, Menzies-Gow A, et al. Predictors of enhanced response with benralizumab for patients with severe asthma: pooled analysis of the SIROCCO and CALIMA studies. *Lancet Respir Med* 2018; **6**(1): 51-64.
101. Anderson WJ, Short PM, Williamson PA, Lipworth BJ. Inhaled corticosteroid dose response using domiciliary exhaled nitric oxide in persistent asthma: the FENOtype trial. *Chest* 2012; **142**(6): 1553-61.
102. Heaney LG, Busby J, Bradding P, et al. Remotely Monitored Therapy and Nitric Oxide Suppression Identifies Nonadherence in Severe Asthma. *Am J Respir Crit Care Med* 2019; **199**(4): 454-64.
103. Fowler SJ, Tavernier G, Niven R. High blood eosinophil counts predict sputum eosinophilia in patients with severe asthma. *J Allergy Clin Immunol* 2015; **135**(3): 822-4.e2.
104. Mukherjee M, Nair P. Blood or sputum eosinophils to guide asthma therapy? *Lancet Respir Med* 2015; **3**(11): 824-5.
105. Lipworth B, Chan R, Kuo CR. Omalizumab or dupilumab for chronic rhinosinusitis with nasal polyposis. *J Allergy Clin Immunol* 2021; **147**(1): 413.
106. Lehtimäki L, Shrimanker R, Moran A, et al. P13 Exhaled nitric oxide and blood eosinophil count in predicting sputum inflammatory type in a heterogeneous airways disease population. *Thorax* 2019; **74**(Suppl 2): A95-A.
107. Gao J, Wu F. Association between fractional exhaled nitric oxide, sputum induction and peripheral blood eosinophil in uncontrolled asthma. *Allergy Asthma Clin Immunol* 2018; **14**: 21.
108. Svenningsen S, Haider E, Boylan C, et al. CT and Functional MRI to Evaluate Airway Mucus in Severe Asthma. *Chest* 2019; **155**(6): 1178-89.
109. Busse W, Chupp G, Nagase H, et al. Anti-IL-5 treatments in patients with severe asthma by blood eosinophil thresholds: Indirect treatment comparison. *J Allergy Clin Immunol* 2019; **143**(1): 190-200.e20.
110. Bourdin A, Husereau D, Molinari N, et al. Matching-adjusted indirect comparison of benralizumab versus interleukin-5 inhibitors for the treatment of severe asthma: a systematic review. *Eur Respir J* 2018; **52**(5).

111. Kavanagh J, Roxas C, Green L, et al. S53 Response to benralizumab after sub-optimal response to mepolizumab in severe eosinophilic asthma. *Thorax* 2019; **74**(Suppl 2): A36-A7.
112. Corren J, Parnes JR, Wang L, et al. Tezepelumab in Adults with Uncontrolled Asthma. *New England Journal of Medicine* 2017; **377**(10): 936-46.
113. Rupani H, Hew M. Super-Responders to Severe Asthma Treatments: Defining a New Paradigm. *J Allergy Clin Immunol Pract* 2021; **9**(11): 4005-6.
114. Upham JW, Le Lievre C, Jackson DJ, Masoli M, Wechsler ME, Price DB. Defining a Severe Asthma Super-Responder: Findings from a Delphi Process. *J Allergy Clin Immunol Pract* 2021; **9**(11): 3997-4004.
115. Juniper EF, O'Byrne PM, Roberts JN. Measuring asthma control in group studies: do we need airway calibre and rescue beta2-agonist use? *Respir Med* 2001; **95**(5): 319-23.
116. Tamari M, Trier AM, Kim BS. Emerging targeted therapeutics underscore immunologic heterogeneity of asthma. *Journal of Allergy and Clinical Immunology* 2021; **148**(3): 719-21.
117. Omalizumab for treating severe persistent allergic asthma. National Institute for Health and Care Excellence Guidelines. https://www.nice.org.uk/guidance/ta278 Date Accessed: November 6.
118. Mepolizumab for treating severe eosinophilic asthma. National Institute for Health and Care Excellence Guidelines. https://www.nice.org.uk/guidance/ta671 Date Accessed November 6.
119. Benralizumab for treating severe eosinophilic asthma. National Institute for Health and Care Excellence Guidelines. https://www.nice.org.uk/guidance/ta565 Date Accessed November 6.
120. Reslizumab for treating severe eosinophilic asthma. National Institute for Health and Care Excellence Guidelines. https://www.nice.org.uk/guidance/ta479 Date Accessed November 6.
121. Dupilumab for treating severe asthma [ID1213]. National Institute for Health and Care Excellence. https://www.nice.org.uk/guidance/indevelopment/gid-ta10276 Date Accessed November 6.
122. Dupilumab (Dupixent). Scottish Medicines Consortium Guidelines. https://www.scottishmedicines.org.uk/medicines-advice/dupilumab-dupixent-full-smc2317/ Date Accessed November 6.
123. Rodrigo GJ, Neffen H, Castro-Rodriguez JA. Efficacy and Safety of Subcutaneous Omalizumab vs Placebo as Add-on Therapy to Corticosteroids for Children and Adults With Asthma: A Systematic Review. *Chest* 2011; **139**(1): 28-35.
124. Zayed Y, Kheiri B, Banifadel M, et al. Dupilumab safety and efficacy in uncontrolled asthma: a systematic review and meta-analysis of randomized clinical trials. *Journal of Asthma* 2019; **56**(10): 1110-9.
125. Nair P, Wenzel S, Rabe KF, et al. Oral Glucocorticoid–Sparing Effect of Benralizumab in Severe Asthma. *New England Journal of Medicine* 2017; **376**(25): 2448-58.
126. Bel EH, Wenzel SE, Thompson PJ, et al. Oral glucocorticoid-sparing effect of mepolizumab in eosinophilic asthma. *N Engl J Med* 2014; **371**(13): 1189-97.
127. Rabe KF, Nair P, Brusselle G, et al. Efficacy and Safety of Dupilumab in Glucocorticoid-Dependent Severe Asthma. *N Engl J Med* 2018; **378**(26): 2475-85.
128. Menzies-Gow A, Gurnell M, Heaney LG, et al. Oral corticosteroid elimination via a personalised reduction algorithm in adults with severe, eosinophilic asthma treated with benralizumab (PONENTE): a multicentre, open-label, single-arm study. *The Lancet Respiratory Medicine*.

129. Diver S, Khalfaoui L, Emson C, et al. Effect of tezepelumab on airway inflammatory cells, remodelling, and hyperresponsiveness in patients with moderate-to-severe uncontrolled asthma (CASCADE): a double-blind, randomised, placebo-controlled, phase 2 trial. *Lancet Respir Med* 2021.
130. Gauvreau GM, Sehmi R, Ambrose CS, Griffiths JM. Thymic stromal lymphopoietin: its role and potential as a therapeutic target in asthma. *Expert Opin Ther Targets* 2020; **24**(8): 777-92.
131. Kitajima M, Lee HC, Nakayama T, Ziegler SF. TSLP enhances the function of helper type 2 cells. *Eur J Immunol* 2011; **41**(7): 1862-71.
132. Menzies-Gow A, Corren J, Bourdin A, et al. Tezepelumab in Adults and Adolescents with Severe, Uncontrolled Asthma. *N Engl J Med* 2021; **384**(19): 1800-9.
133. Tanaka J, Watanabe N, Kido M, et al. Human TSLP and TLR3 ligands promote differentiation of Th17 cells with a central memory phenotype under Th2-polarizing conditions. *Clin Exp Allergy* 2009; **39**(1): 89-100.
134. Liang Y, Yu B, Chen J, et al. Thymic stromal lymphopoietin epigenetically upregulates Fc receptor γ subunit-related receptors on antigen-presenting cells and induces T(H)2/T(H)17 polarization through dectin-2. *J Allergy Clin Immunol* 2019; **144**(4): 1025-35.e7.
135. Gow AM, Brightling CE, Kuna P, et al. Oral Corticosteroid-Sparing Effect of Tezepelumab in Adults with Severe Asthma. D7 D007 ADVANCES IN ASTHMA THERAPIES: A1197-A.
136. A. Menzies-Gow CEB, C.S. Ambrose, B. Cook, Hellqvist, J.-P. Llanos Ackert, G. Colice and M. Wechsler Effect of Tezepelumab in Oral Corticosteroid-Dependent Patients with Severe Asthma: Results From the Phase 3 NAVIGATOR Study. *American Journal of Respiratory and Critical Care Medicine 2021;203:A1442*.
137. Couillard S, Shrimanker R, Chaudhuri R, et al. Fractional Exhaled Nitric Oxide Nonsuppression Identifies Corticosteroid-Resistant Type 2 Signaling in Severe Asthma. *Am J Respir Crit Care Med* 2021; **204**(6): 731-4.
138. Manson ML, Säfholm J, James A, et al. IL-13 and IL-4, but not IL-5 nor IL-17A, induce hyperresponsiveness in isolated human small airways. *J Allergy Clin Immunol* 2020; **145**(3): 808-17.e2.
139. Sverrild A, Hansen S, Hvidtfeldt M, et al. The effect of tezepelumab on airway hyperresponsiveness to mannitol in asthma (UPSTREAM). *European Respiratory Journal* 2021: 2101296.
140. Gauvreau G, Hohlfeld J, Boulet L-P, et al. Late Breaking Abstract - Efficacy of CSJ117 on allergen-induced asthmatic responses in mild atopic asthma patients. *European Respiratory Journal* 2020; **56**(suppl 64): 3690.
141. Préfontaine D, Nadigel J, Chouiali F, et al. Increased IL-33 expression by epithelial cells in bronchial asthma. *Journal of Allergy and Clinical Immunology* 2010; **125**(3): 752-4.
142. Préfontaine D, Lajoie-Kadoch S, Foley S, et al. Increased Expression of IL-33 in Severe Asthma: Evidence of Expression by Airway Smooth Muscle Cells. *The Journal of Immunology* 2009; **183**(8): 5094-103.
143. Cherry WB, Yoon J, Bartemes KR, Iijima K, Kita H. A novel IL-1 family cytokine, IL-33, potently activates human eosinophils. *Journal of Allergy and Clinical Immunology* 2008; **121**(6): 1484-90.
144. Kelsen SG, Agache IO, Soong W, et al. Astegolimab (anti-ST2) efficacy and safety in adults with severe asthma: A randomized clinical trial. *J Allergy Clin Immunol* 2021; **148**(3): 790-8.

145. Wechsler ME, Ruddy MK, Pavord ID, et al. Efficacy and Safety of Itepekimab in Patients with Moderate-to-Severe Asthma. *N Engl J Med* 2021; **385**(18): 1656-68.
146. Salter BM, Oliveria JP, Nusca G, et al. IL-25 and IL-33 induce Type 2 inflammation in basophils from subjects with allergic asthma. *Respir Res* 2016; **17**: 5.
147. Corrigan CJ, Wang W, Meng Q, et al. Allergen-induced expression of IL-25 and IL-25 receptor in atopic asthmatic airways and late-phase cutaneous responses. *Journal of Allergy and Clinical Immunology* 2011; **128**(1): 116-24.
148. Corrigan CJ, Wang W, Meng Q, et al. T-helper cell type 2 (Th2) memory T cell-potentiating cytokine IL-25 has the potential to promote angiogenesis in asthma. *Proc Natl Acad Sci U S A* 2011; **108**(4): 1579-84.
149. Deng C, Peng N, Tang Y, et al. Roles of IL-25 in Type 2 Inflammation and Autoimmune Pathogenesis. *Front Immunol* 2021; **12**: 691559-.
150. Busse WW, Holgate S, Kerwin E, et al. Randomized, double-blind, placebo-controlled study of brodalumab, a human anti-IL-17 receptor monoclonal antibody, in moderate to severe asthma. *Am J Respir Crit Care Med* 2013; **188**(11): 1294-302.
151. Briegel I, Felicio-Briegel A, Mertsch P, Kneidinger N, Haubner F, Milger K. Hypereosinophilia with systemic manifestations under dupilumab and possibility of dual benralizumab and dupilumab therapy in patients with asthma and CRSwNP. *J Allergy Clin Immunol Pract* 2021.
152. Wu EY, Hernandez ML, Jennette JC, Falk RJ. Eosinophilic Granulomatosis with Polyangiitis: Clinical Pathology Conference and Review. *The Journal of Allergy and Clinical Immunology: In Practice* 2018; **6**(5): 1496-504.
153. Khatri SB, Iaccarino JM, Barochia A, et al. Use of Fractional Exhaled Nitric Oxide to Guide the Treatment of Asthma: An Official American Thoracic Society Clinical Practice Guideline. *Am J Respir Crit Care Med* 2021; **204**(10): e97-e109.
154. Benralizumab (Fasenra). Scottish Medicines Consortium Guideline. https://www.scottishmedicines.org.uk/medicines-advice/benralizumab-fasenra-fullsubmission-smc2155/ Date accessed November 17.
155. Postma DS, Brightling C, Baldi S, et al. Exploring the relevance and extent of small airways dysfunction in asthma (ATLANTIS): baseline data from a prospective cohort study. *Lancet Respir Med* 2019; **7**(5): 402-16.
156. van der Wiel E, ten Hacken NH, Postma DS, van den Berge M. Small-airways dysfunction associates with respiratory symptoms and clinical features of asthma: a systematic review. *J Allergy Clin Immunol* 2013; **131**(3): 646-57.
157. Chan R, Misirovs R, Lipworth B. Repeatability of impulse oscillometry in patients with severe asthma. *Eur Respir J* 2021.
158. Foy BH, Soares M, Bordas R, et al. Lung Computational Models and the Role of the Small Airways in Asthma. *Am J Respir Crit Care Med* 2019; **200**(8): 982-91.
159. Chan R, RuiWen Kuo C, Lipworth B. Real-life small airway outcomes in severe asthma patients receiving biologic therapies. *J Allergy Clin Immunol Pract* 2021; **9**(7): 2907-9.
160. Pelaia C, Busceti MT, Crimi C, et al. Real-Life effects of benralizumab on exacerbation number and lung hyperinflation in atopic patients with severe eosinophilic asthma. *Biomed Pharmacother* 2020; **129**: 110444.
161. Kolkhir P, Altrichter S, Munoz M, Hawro T, Maurer M. New treatments for chronic urticaria. *Ann Allergy Asthma Immunol* 2020; **124**(1): 2-12.
162. Greuter T, Hirano I, Dellon ES. Emerging therapies for eosinophilic esophagitis. *J Allergy Clin Immunol* 2020; **145**(1): 38-45.

163. Ratchataswan T, Banzon TM, Thyssen JP, Weidinger S, Guttman-Yassky E, Phipatanakul W. Biologics for Treatment of Atopic Dermatitis: Current Status and Future Prospect. *J Allergy Clin Immunol Pract* 2021; **9**(3): 1053-65.
164. Lipworth BJ, Chan R. The Choice of Biologics in Patients with Severe Chronic Rhinosinusitis with Nasal Polyps. *J Allergy Clin Immunol Pract* 2021.
165. Oykhman P, Paramo FA, Bousquet J, Kennedy DW, Brignardello-Petersen R, Chu DK. Comparative efficacy and safety of monoclonal antibodies and aspirin desensitization for chronic rhinosinusitis with nasal polyposis: A systematic review and network meta-analysis. *J Allergy Clin Immunol* 2021; **10.1016/j.jaci.2021.09.009**.
166. Dupixent prescribing information. https://www.regeneron.com/downloads/dupixent_fpi.pdf Accessed November 16.
167. Eger K, Pet L, Weersink EJM, Bel EH. Complications of switching from anti-IL-5 or anti-IL-5R to dupilumab in corticosteroid-dependent severe asthma. *J Allergy Clin Immunol Pract* 2021; **9**(7): 2913-5.
168. Laviolette M, Gossage DL, Gauvreau G, et al. Effects of benralizumab on airway eosinophils in asthmatic patients with sputum eosinophilia. *Journal of Allergy and Clinical Immunology* 2013; **132**(5): 1086-96.e5.
169. Drick N, Milger K, Seeliger B, et al. Switch from IL-5 to IL-5-Receptor α Antibody Treatment in Severe Eosinophilic Asthma. *Journal of asthma and allergy* 2020; **13**: 605-14.
170. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J* 2005; **26**(2): 319-38.
171. Oostveen E, MacLeod D, Lorino H, et al. The forced oscillation technique in clinical practice: methodology, recommendations and future developments. *Eur Respir J* 2003; **22**(6): 1026-41.
172. Anderson SD, Brannan J, Spring J, et al. A new method for bronchial-provocation testing in asthmatic subjects using a dry powder of mannitol. *Am J Respir Crit Care Med* 1997; **156**(3 Pt 1): 758-65.
173. Juniper EF, Buist AS, Cox FM, Ferrie PJ, King DR. Validation of a standardized version of the Asthma Quality of Life Questionnaire. *Chest* 1999; **115**(5): 1265-70.
174. Juniper EF, Guyatt GH, Willan A, Griffith LE. Determining a minimal important change in a disease-specific Quality of Life Questionnaire. *J Clin Epidemiol* 1994; **47**(1): 81-7.
175. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. *Eur Respir J* 1999; **14**(4): 902-7.
176. Juniper EF, Bousquet J, Abetz L, Bateman ED. Identifying 'well-controlled' and 'not well-controlled' asthma using the Asthma Control Questionnaire. *Respir Med* 2006; **100**(4): 616-21.
177. Berger WE, White MV. Efficacy of azelastine nasal spray in patients with an unsatisfactory response to loratadine. *Annals of Allergy, Asthma & Immunology* 2003; **91**(2): 205-11.
178. Mo S, Gupta SS, Stroud A, et al. Nasal Peak Inspiratory Flow in Healthy and Obstructed Patients: Systematic Review and Meta-Analysis. *Laryngoscope* 2021; **131**(2): 260-7.
179. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005; **171**(8): 912-30.
180. Dunican EM, Elicker BM, Gierada DS, et al. Mucus plugs in patients with asthma linked to eosinophilia and airflow obstruction. *The Journal of Clinical Investigation* 2018; **128**(3): 997-1009.

181. Lipworth B, Manoharan A, Anderson W. Unlocking the quiet zone: the small airway asthma phenotype. *Lancet Respir Med* 2014; **2**(6): 497-506.
182. Chan R, Lipworth B. Determinants of asthma control and exacerbations in moderate to severe asthma. *The Journal of Allergy and Clinical Immunology: In Practice*.
183. Chiu HY, Hsiao YH, Su KC, Lee YC, Ko HK, Perng DW. Small Airway Dysfunction by Impulse Oscillometry in Symptomatic Patients with Preserved Pulmonary Function. *J Allergy Clin Immunol Pract* 2020; **8**(1): 229-35.e3.
184. Cottini M, Licini A, Lombardi C, Berti A. Clinical Characterization and Predictors of IOS-Defined Small-Airway Dysfunction in Asthma. *The Journal of Allergy and Clinical Immunology: In Practice* 2020; **8**(3): 997-1004.e2.
185. Kuo CR, Jabbal S, Lipworth B. Is small airways dysfunction related to asthma control and type 2 inflammation? *Ann Allergy Asthma Immunol* 2018; **121**(5): 631-2.
186. Jabbal S, Manoharan A, Lipworth J, Lipworth B. Utility of impulse oscillometry in patients with moderate to severe persistent asthma. *J Allergy Clin Immunol* 2016; **138**(2): 601-3.
187. Chan R, Lipworth BJ. Combining low-frequency oscillometry and spirometry measurements in relation to asthma control and exacerbations in moderate-to-severe asthma. *J Allergy Clin Immunol Pract* 2022.
188. Chan R, Lipworth B. Forced Vital Capacity and Low Frequency Reactance Area Measurements Are Associated with Asthma Control and Exacerbations. *Lung* 2022; **200**(3): 301-3.
189. Robinson PD, Latzin P, Verbanck S, et al. Consensus statement for inert gas washout measurement using multiple- and single- breath tests. *Eur Respir J* 2013; **41**(3): 507-22.
190. Berry M, Hargadon B, Morgan A, et al. Alveolar nitric oxide in adults with asthma: evidence of distal lung inflammation in refractory asthma. *Eur Respir J* 2005; **25**(6): 986-91.
191. Martin RJ. Therapeutic significance of distal airway inflammation in asthma. *J Allergy Clin Immunol* 2002; **109**(2 Suppl): S447-60.
192. Williamson PA, Short PM, Vaidyanathan S, Lipworth BJ. Inhaled and systemic corticosteroid response in severe asthma assessed by alveolar nitric oxide: a randomized crossover pilot study of add-on therapy. *Br J Clin Pharmacol* 2013; **75**(1): 93-102.
193. Weiler CR. Omalizumab and Mast Cell Disorders: Are We There Yet? *The Journal of Allergy and Clinical Immunology: In Practice* 2019; **7**(7): 2396-7.
194. Chang TW. The pharmacological basis of anti-IgE therapy. *Nature Biotechnology* 2000; **18**(2): 157-62.
195. Den Otter I, Silva LFF, Carvalho ALN, et al. High-affinity immunoglobulin E receptor expression is increased in large and small airways in fatal asthma. *Clinical & Experimental Allergy* 2010; **40**(10): 1473-81.
196. Huang W-C, Fu P-K, Chan M-C, et al. The Long-Term Effectiveness of Omalizumab in Adult Patients with Severe Allergic Asthma: Continuous Treatment Versus Boosting Treatment. *Journal of Clinical Medicine* 2021; **10**(4): 707.
197. Tajiri T, Niimi A, Matsumoto H, et al. Comprehensive efficacy of omalizumab for severe refractory asthma: a time-series observational study. *Annals of Allergy, Asthma & Immunology* 2014; **113**(4): 470-5.e2.
198. Bjermer L. The role of small airway disease in asthma. *Curr Opin Pulm Med* 2014; **20**(1): 23-30.
199. Cameli P, Perruzza M, Salvini M, et al. Omalizumab treatment in Samter's triad: case series and review of the literature. *Eur Rev Med Pharmacol Sci* 2019; **23**(18): 8124-9.

200. Pelaia C, Vatrella A, Busceti MT, et al. Severe eosinophilic asthma: from the pathogenic role of interleukin-5 to the therapeutic action of mepolizumab. *Drug Des Devel Ther* 2017; **11**: 3137-44.
201. Pelaia G, Vatrella A, Busceti MT, et al. Role of biologics in severe eosinophilic asthma - focus on reslizumab. *Ther Clin Risk Manag* 2016; **12**: 1075-82.
202. Pelaia C, Calabrese C, Vatrella A, et al. Benralizumab: From the Basic Mechanism of Action to the Potential Use in the Biological Therapy of Severe Eosinophilic Asthma. *Biomed Res Int* 2018; **2018**: 4839230.
203. Tulic MK, Christodoulopoulos P, Hamid Q. Small airway inflammation in asthma. *Respiratory Research* 2001; **2**(6): 333.
204. Chupp GL, Bradford ES, Albers FC, et al. Efficacy of mepolizumab add-on therapy on health-related quality of life and markers of asthma control in severe eosinophilic asthma (MUSCA): a randomised, double-blind, placebo-controlled, parallel-group, multicentre, phase 3b trial. *The Lancet Respiratory Medicine* 2017; **5**(5): 390-400.
205. Sposato B, Camiciottoli G, Bacci E, et al. Mepolizumab effectiveness on small airway obstruction, corticosteroid sparing and maintenance therapy step-down in real life. *Pulm Pharmacol Ther* 2020; **61**: 101899.
206. Maglio A, Vitale C, Pellegrino S, et al. Real-Life Effectiveness of Mepolizumab on Forced Expiratory Flow between 25% and 75% of Forced Vital Capacity in Patients with Severe Eosinophilic Asthma. *Biomedicines* 2021; **9**(11).
207. Yılmaz İ, Nazik Bahçecioğlu S, Türk M, Tutar N, Paçacı Çetin G, Arslan B. Effectiveness of mepolizumab therapy on symptoms, asthma exacerbations, steroid dependence, and small airways in patients with severe eosinophilic asthma. *Turk J Med Sci* 2021; **51**(4): 1953-9.
208. Antonicelli L, Tontini C, Marchionni A, Lucchetti B, Garritani MS, Bilò MB. Forced oscillation technique as method to document and monitor the efficacy of mepolizumab in treating severe eosinophilic asthma. *Allergy* 2020; **75**(2): 433-6.
209. Farah CS, Badal T, Reed N, et al. Mepolizumab improves small airway function in severe eosinophilic asthma. *Respir Med* 2019; **148**: 49-53.
210. Bjermer L, Lemiere C, Maspero J, Weiss S, Zangrilli J, Germinaro M. Reslizumab for Inadequately Controlled Asthma With Elevated Blood Eosinophil Levels: A Randomized Phase 3 Study. *Chest* 2016; **150**(4): 789-98.
211. Murphy K, Jacobs J, Bjermer L, et al. Long-term Safety and Efficacy of Reslizumab in Patients with Eosinophilic Asthma. *The Journal of Allergy and Clinical Immunology: In Practice* 2017; **5**(6): 1572-81.e3.
212. Virchow JC, Hickey L, Du E, Garin M. In patients with severe asthma with eosinophilia in reslizumab clinical trials, high peripheral blood eosinophil levels are associated with low FEV(1) reversibility. *Allergy Asthma Clin Immunol* 2020; **16**: 26.
213. Nolasco S, Crimi C, Pelaia C, et al. Benralizumab Effectiveness in Severe Eosinophilic Asthma with and without Chronic Rhinosinusitis with Nasal Polyps: A Real-World Multicenter Study. *J Allergy Clin Immunol Pract* 2021; **9**(12): 4371-80.e4.
214. Shirai T, Akamatsu T, Hirai K, et al. Oscillometry improves earlier than spirometry after benralizumab initiation in severe asthma. *Allergy* 2020; **75**(10): 2678-80.
215. Harb H, Chatila TA. Mechanisms of Dupilumab. *Clinical & Experimental Allergy* 2020; **50**(1): 5-14.
216. Castro M, Rabe KF, Corren J, et al. Dupilumab improves lung function in patients with uncontrolled, moderate-to-severe asthma. *ERJ Open Res* 2020; **6**(1).

217. Pelaia C, Lombardo N, Busceti MT, et al. Short-Term Evaluation of Dupilumab Effects in Patients with Severe Asthma and Nasal Polyposis. *J Asthma Allergy* 2021; **14**: 1165-72.
218. Minagawa S, Araya J, Watanabe N, et al. Real-life effectiveness of dupilumab in patients with mild to moderate bronchial asthma comorbid with CRSwNP. *BMC Pulm Med* 2022; **22**(1): 258.
219. Marone G, Spadaro G, Braile M, et al. Tezepelumab: a novel biological therapy for the treatment of severe uncontrolled asthma. *Expert Opin Investig Drugs* 2019; **28**(11): 931-40.
220. Manoharan A, Anderson WJ, Lipworth J, Ibrahim I, Lipworth BJ. Small airway dysfunction is associated with poorer asthma control. *Eur Respir J* 2014; **44**(5): 1353-5.
221. Heffler E, Crimi C, Campisi R, et al. Bronchodilator response as a marker of poor asthma control. *Respir Med* 2016; **112**: 45-50.
222. Puckett JL, Taylor RW, Leu SY, et al. An elevated bronchodilator response predicts large airway inflammation in mild asthma. *Pediatr Pulmonol* 2010; **45**(2): 174-81.
223. Szefler SJ, Martin RJ, King TS, et al. Significant variability in response to inhaled corticosteroids for persistent asthma. *J Allergy Clin Immunol* 2002; **109**(3): 410-8.
224. Kuo CR, Chan R, Lipworth B. Impulse oscillometry bronchodilator response and asthma control. *J Allergy Clin Immunol Pract* 2020; **8**(10): 3610-2.
225. Short PM, Williamson PA, Lipworth BJ. Sensitivity of impulse oscillometry and spirometry in beta-blocker induced bronchoconstriction and beta-agonist bronchodilatation in asthma. *Ann Allergy Asthma Immunol* 2012; **109**(6): 412-5.
226. Johansson H, Wollmer P, Sundström J, Janson C, Malinovschi A. Bronchodilator response in FOT parameters in middle-aged adults from SCAPIS: normal values and relationship to asthma and wheezing. *European Respiratory Journal* 2021; **58**(3): 2100229.
227. Jang AS, Lee JH, Park SW, Park JS, Kim DJ, Park CS. Risk factors related to fixed airway obstruction in patients with asthma after antiasthma treatment. *Ann Allergy Asthma Immunol* 2007; **99**(5): 408-12.
228. Cottee AM, Seccombe LM, Thamrin C, King GG, Peters MJ, Farah CS. Bronchodilator Response Assessed by the Forced Oscillation Technique Identifies Poor Asthma Control With Greater Sensitivity Than Spirometry. *Chest* 2020; **157**(6): 1435-41.
229. Grove A, Lipworth BJ. Bronchodilator subsensitivity to salbutamol after twice daily salmeterol in asthmatic patients. *Lancet* 1995; **346**(8969): 201-6.
230. Lee DK, Jackson CM, Currie GP, Cockburn WJ, Lipworth BJ. Comparison of combination inhalers vs inhaled corticosteroids alone in moderate persistent asthma. *Br J Clin Pharmacol* 2003; **56**(5): 494-500.
231. Bednarek M, Grabicki M, Piorunek T, Batura-Gabryel H. “Current place of impulse oscillometry in the assessment of pulmonary diseases.”. *Respiratory Medicine* 2020; **170**: 105952.
232. Tian L. Inferences on the common coefficient of variation. *Stat Med* 2005; **24**(14): 2213-20.
233. Pennock BE, Rogers RM, McCaffree DR. Changes in Measured Spirometric Indices: What Is Significant? *CHEST* 1981; **80**(1): 97-9.
234. Graham BL, Steenbruggen I, Miller MR, et al. Standardization of Spirometry 2019 Update. An Official American Thoracic Society and European Respiratory Society Technical Statement. *Am J Respir Crit Care Med* 2019; **200**(8): e70-e88.
235. Goldman MD, Carter R, Klein R, Fritz G, Carter B, Pachucki P. Within- and between-day variability of respiratory impedance, using impulse oscillometry in adolescent asthmatics. *Pediatr Pulmonol* 2002; **34**(4): 312-9.

236. Cottee AM, Seccombe LM, Thamrin C, et al. Longitudinal monitoring of asthma in the clinic using respiratory oscillometry. *Respirology* 2021; **26**(6): 566-73.
237. Takeda T, Oga T, Niimi A, et al. Relationship between small airway function and health status, dyspnea and disease control in asthma. *Respiration* 2010; **80**(2): 120-6.
238. Mead J. The lung's "quiet zone". *N Engl J Med* 1970; **282**(23): 1318-9.
239. Chan R, Kuo C, Lipworth B. A retrospective cohort study to evaluate the relationship of airway hyperresponsiveness to type 2 biomarkers in persistent asthma. *Arch Asthma Allergy Immunol 2021; 5: 008-013*.
240. Riley CM, Wenzel SE, Castro M, et al. Clinical Implications of Having Reduced Mid Forced Expiratory Flow Rates (FEF25-75), Independently of FEV1, in Adult Patients with Asthma. *PLoS One* 2015; **10**(12): e0145476.
241. Almeshari MA, Alobaidi NY, Edgar RG, Stockley J, Sapey E. Physiological tests of small airways function in diagnosing asthma: a systematic review. *BMJ Open Respir Res* 2020; **7**(1).
242. Meltzer EO, Busse WW, Wenzel SE, et al. Use of the Asthma Control Questionnaire to predict future risk of asthma exacerbation. *J Allergy Clin Immunol* 2011; **127**(1): 167-72.
243. Vaidyanathan S, Barnes M, Williamson P, Hopkinson P, Donnan PT, Lipworth B. Treatment of Chronic Rhinosinusitis With Nasal Polyposis With Oral Steroids Followed by Topical Steroids: A Randomized Trial. *Annals of Internal Medicine* 2011; **154**(5): 293-302.
244. Fokkens WJ, Lund VJ, Hopkins C, et al. European Position Paper on Rhinosinusitis and Nasal Polyps 2020. *Rhinology* 2020; **58**(Suppl S29): 1-464.
245. Laidlaw TM, Mullol J, Woessner KM, Amin N, Mannent LP. Chronic Rhinosinusitis with Nasal Polyps and Asthma. *J Allergy Clin Immunol Pract* 2021; **9**(3): 1133-41.
246. Bachert C, Sousa AR, Lund VJ, et al. Reduced need for surgery in severe nasal polyposis with mepolizumab: Randomized trial. *J Allergy Clin Immunol* 2017; **140**(4): 1024-31.e14.
247. Gevaert P, Van Bruaene N, Cattaert T, et al. Mepolizumab, a humanized anti-IL-5 mAb, as a treatment option for severe nasal polyposis. *J Allergy Clin Immunol* 2011; **128**(5): 989-95.e1-8.
248. Langdon C, Mullol J. Nasal polyps in patients with asthma: prevalence, impact, and management challenges. *J Asthma Allergy* 2016; **9**: 45-53.
249. Klossek JM, Neukirch F, Pribil C, et al. Prevalence of nasal polyposis in France: a cross-sectional, case-control study. *Allergy* 2005; **60**(2): 233-7.
250. Larsen K. The clinical relationship of nasal polyps to asthma. *Allergy Asthma Proc* 1996; **17**(5): 243-9.
251. Wu D, Bleier BS, Wei Y. Progression from nasal polyps to adult-onset asthma: a different process from atopic march? ; 2018; 2018.
252. Stevens WW, Peters AT, Hirsch AG, et al. Clinical Characteristics of Patients with Chronic Rhinosinusitis with Nasal Polyps, Asthma, and Aspirin-Exacerbated Respiratory Disease. *J Allergy Clin Immunol Pract* 2017; **5**(4): 1061-70.e3.
253. Gevaert P, Omachi TA, Corren J, et al. Efficacy and safety of omalizumab in nasal polyposis: 2 randomized phase 3 trials. *J Allergy Clin Immunol* 2020; **146**(3): 595-605.
254. Han JK, Bachert C, Fokkens W, et al. Mepolizumab for chronic rhinosinusitis with nasal polyps (SYNAPSE): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Respir Med* 2021.
255. Bachert C, Han JK, Desrosiers MY, et al. Efficacy and Safety of Benralizumab in Chronic Rhinosinusitis with Nasal Polyps: A Randomized, Placebo-controlled Trial. *J Allergy Clin Immunol* 2021.

256. Ragab SM, Lund VJ, Saleh HA, Scadding G. Nasal nitric oxide in objective evaluation of chronic rhinosinusitis therapy. *Allergy* 2006; **61**(6): 717-24.
257. Fokkens WJ, Lund V, Bachert C, et al. EUFOREA consensus on biologics for CRSwNP with or without asthma. *Allergy* 2019; **74**(12): 2312-9.
258. Maio S, Baldacci S, Bresciani M, et al. RItA: The Italian severe/uncontrolled asthma registry. *Allergy* 2018; **73**(3): 683-95.
259. Zhang Y, Derycke L, Holtappels G, et al. Th2 cytokines orchestrate the secretion of MUC5AC and MUC5B in IL-5-positive chronic rhinosinusitis with nasal polyps. *Allergy* 2019; **74**(1): 131-40.
260. Peters AT, Han JK, Hellings P, et al. Indirect Treatment Comparison of Biologics in Chronic Rhinosinusitis with Nasal Polyps. *J Allergy Clin Immunol Pract* 2021.
261. Kuo CR, Chan R, Lipworth B. Does unified allergic airway disease impact on lung function and type 2 biomarkers? *Allergy, Asthma & Clinical Immunology* 2019; **15**(1): 75.
262. Du K, Zheng M, Zhao Y, et al. Impaired small airway function in non-asthmatic chronic rhinosinusitis with nasal polyps. *Clinical & Experimental Allergy* 2020; **50**(12): 1362-71.
263. Kuo CR, Jabbal S, Lipworth B. I Say IOS You Say AOS: Comparative Bias in Respiratory Impedance Measurements. *Lung* 2019; **197**(4): 473-81.
264. Bateman ED, Reddel HK, Eriksson G, et al. Overall asthma control: the relationship between current control and future risk. *J Allergy Clin Immunol* 2010; **125**(3): 600-8, 8.e1-8.e6.
265. Laidlaw TM, Mullol J, Fan C, et al. Dupilumab improves nasal polyp burden and asthma control in patients with CRSwNP and AERD. *J Allergy Clin Immunol Pract* 2019; **7**(7): 2462-5.e1.
266. Coumou H, Westerhof GA, de Nijs SB, Zwinderman AH, Bel EH. Predictors of accelerated decline in lung function in adult-onset asthma. *Eur Respir J* 2018; **51**(2).
267. Chan R, RuiWen Kuo C, Lipworth B. Real life small airway outcomes in severe asthma patients receiving biologic therapies. *The Journal of Allergy and Clinical Immunology: In Practice*.
268. FitzGerald JM, Bleecker ER, Nair P, et al. Benralizumab, an anti-interleukin-5 receptor α monoclonal antibody, as add-on treatment for patients with severe, uncontrolled, eosinophilic asthma (CALIMA): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet* 2016; **388**(10056): 2128-41.
269. Jabbal S, Lipworth BJ. Blood eosinophils: The forgotten man of inhaled steroid dose titration. *Clin Exp Allergy* 2018; **48**(1): 93-5.
270. Maniscalco M, Weitzberg E, Sundberg J, Sofia M, Lundberg JO. Assessment of nasal and sinus nitric oxide output using single-breath humming exhalations. *European Respiratory Journal* 2003; **22**(2): 323-9.
271. Wenzel S. Severe asthma in adults. *Am J Respir Crit Care Med* 2005; **172**(2): 149-60.
272. Paganin F, Séneterre E, Chanez P, et al. Computed tomography of the lungs in asthma: influence of disease severity and etiology. *Am J Respir Crit Care Med* 1996; **153**(1): 110-4.
273. Paganin F, Trussard V, Seneterre E, et al. Chest radiography and high resolution computed tomography of the lungs in asthma. *Am Rev Respir Dis* 1992; **146**(4): 1084-7.
274. Gupta S, Siddiqui S, Haldar P, et al. Qualitative analysis of high-resolution CT scans in severe asthma. *Chest* 2009; **136**(6): 1521-8.
275. Glynn AA, Michaels L. Bronchial Biopsy in Chronic Bronchitis and Asthma. *Thorax* 1960; **15**(2): 142-53.
276. Houston JC, De Navasquez S, Trounce JR. A clinical and pathological study of fatal cases of status asthmaticus. *Thorax* 1953; **8**(3): 207-13.

277. Tang M, Elicker BM, Henry T, et al. Mucus Plugs Persist in Asthma, and Changes in Mucus Plugs Associate with Changes in Airflow over Time. *Am J Respir Crit Care Med* 2022; **205**(9): 1036-45.
278. Corren J, Du E, Gubbi A, Vanlandingham R. Variability in Blood Eosinophil Counts in Patients with Eosinophilic Asthma. *J Allergy Clin Immunol Pract* 2021; **9**(3): 1224-31.e9.
279. Meltzer EO, Hamilos DL, Hadley JA, et al. Rhinosinusitis: developing guidance for clinical trials. *J Allergy Clin Immunol* 2006; **118**(5 Suppl): S17-61.
280. Patel IS, Vlahos I, Wilkinson TM, et al. Bronchiectasis, exacerbation indices, and inflammation in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2004; **170**(4): 400-7.
281. Tamura K, Shirai T, Hirai K, et al. Mucus Plugs and Small Airway Dysfunction in Asthma, COPD, and Asthma-COPD Overlap. *Allergy Asthma Immunol Res* 2022; **14**(2): 196-209.
282. Hearn AP, Mak MS, Budaj I, et al. The prevalence of mucus plugging in severe eosinophilic asthma and its relationship to clinical efficacy of anti-IL-5R treatment. *J Allergy Clin Immunol Pract* 2022.
283. Tang M, Elicker BM, Henry T, et al. Mucus Plugs Persist in Asthma and Changes in Mucus Plugs Associate with Changes in Airflow Over Time. *American Journal of Respiratory and Critical Care Medicine*; **0**(ja): null.
284. Gudbjartsson T, Gudmundsson G. Middle lobe syndrome: a review of clinicopathological features, diagnosis and treatment. *Respiration* 2012; **84**(1): 80-6.
285. Agarwal R, Chakrabarti A, Shah A, et al. Allergic bronchopulmonary aspergillosis: review of literature and proposal of new diagnostic and classification criteria. *Clin Exp Allergy* 2013; **43**(8): 850-73.
286. Patel G, Greenberger PA. Allergic bronchopulmonary aspergillosis. *Allergy Asthma Proc* 2019; **40**(6): 421-4.
287. Ueki S, Hebisawa A, Kitani M, Asano K, Neves JS. Allergic Bronchopulmonary Aspergillosis-A Luminal Hypereosinophilic Disease With Extracellular Trap Cell Death. *Front Immunol* 2018; **9**: 2346.
288. Oguma T, Asano K, Tomomatsu K, et al. Induction of mucin and MUC5AC expression by the protease activity of Aspergillus fumigatus in airway epithelial cells. *J Immunol* 2011; **187**(2): 999-1005.
289. Menzies D, Holmes L, McCumesky G, Prys-Picard C, Niven R. Aspergillus sensitization is associated with airflow limitation and bronchiectasis in severe asthma. *Allergy* 2011; **66**(5): 679-85.
290. Tomomatsu K, Sugino Y, Okada N, Tanaka J, Oguma T, Asano K. Rapid clearance of mepolizumab-resistant bronchial mucus plugs in allergic bronchopulmonary aspergillosis with benralizumab treatment. *Allergology International* 2020; **69**(4): 636-8.
291. Ekström M, Nwaru BI, Hasvold P, Wiklund F, Telg G, Janson C. Oral corticosteroid use, morbidity and mortality in asthma: A nationwide prospective cohort study in Sweden. *Allergy* 2019; **74**(11): 2181-90.
292. Pietinalho A, Pelkonen A, Rytilä P. Linkage between smoking and asthma. *Allergy* 2009; **64**(12): 1722-7.
293. Chiappara G, Gagliardo R, Siena A, et al. Airway remodelling in the pathogenesis of asthma. *Current Opinion in Allergy and Clinical Immunology* 2001; **1**(1): 85-93.
294. Berair R, Hartley R, Mistry V, et al. Associations in asthma between quantitative computed tomography and bronchial biopsy-derived airway remodelling. *European Respiratory Journal* 2017; **49**(5): 1601507.

295. Niimi A, Matsumoto H, Amitani R, et al. Airway wall thickness in asthma assessed by computed tomography. Relation to clinical indices. *Am J Respir Crit Care Med* 2000; **162**(4 Pt 1): 1518-23.
296. Kasahara K, Shiba K, Ozawa T, Okuda K, Adachi M. Correlation between the bronchial subepithelial layer and whole airway wall thickness in patients with asthma. *Thorax* 2002; **57**(3): 242-6.
297. Awadh N, Müller NL, Park CS, Abboud RT, FitzGerald JM. Airway wall thickness in patients with near fatal asthma and control groups: assessment with high resolution computed tomographic scanning. *Thorax* 1998; **53**(4): 248-53.
298. McDowell KM. Recent Diagnosis Techniques in Pediatric Asthma: Impulse Oscillometry in Preschool Asthma and Use of Exhaled Nitric Oxide. *Immunol Allergy Clin North Am* 2019; **39**(2): 205-19.
299. Majima S, Wakahara K, Nishio T, et al. Bronchial wall thickening is associated with severity of chronic rhinosinusitis. *Respiratory Medicine* 2020; **170**: 106024.
300. Berry H, Huskisson EC. Isotopic indices as a measure of inflammation in rheumatoid arthritis. *Ann Rheum Dis* 1974; **33**(6): 523-5.
301. Kaminsky DA, Simpson SJ, Berger KI, et al. Clinical significance and applications of oscillometry. *European Respiratory Review* 2022; **31**(163): 210208.
302. Boulet L, Bélanger M, Carrier G. Airway responsiveness and bronchial-wall thickness in asthma with or without fixed airflow obstruction. *Am J Respir Crit Care Med* 1995; **152**(3): 865-71.
303. Busse WW. The relationship of airway hyperresponsiveness and airway inflammation: Airway hyperresponsiveness in asthma: its measurement and clinical significance. *Chest* 2010; **138**(2 Suppl): 4s-10s.
304. O'Byrne PM, Inman MD. Airway hyperresponsiveness. *Chest* 2003; **123**(3 Suppl): 411s-6s.
305. Cockcroft DW. Direct challenge tests: Airway hyperresponsiveness in asthma: its measurement and clinical significance. *Chest* 2010; **138**(2 Suppl): 18s-24s.
306. Costello RW, Evans CM, Yost BL, et al. Antigen-induced hyperreactivity to histamine: role of the vagus nerves and eosinophils. *Am J Physiol* 1999; **276**(5): L709-14.
307. Anderson SD. Indirect challenge tests: Airway hyperresponsiveness in asthma: its measurement and clinical significance. *Chest* 2010; **138**(2 Suppl): 25s-30s.
308. Erin EM, Zacharasiewicz AS, Nicholson GC, et al. Rapid effect of inhaled ciclesonide in asthma: a randomized, placebo-controlled study. *Chest* 2008; **134**(4): 740-5.
309. Brightling CE, Bradding P, Symon FA, Holgate ST, Wardlaw AJ, Pavord ID. Mast-cell infiltration of airway smooth muscle in asthma. *N Engl J Med* 2002; **346**(22): 1699-705.
310. Gould HJ, Sutton BJ. IgE in allergy and asthma today. *Nature Reviews Immunology* 2008; **8**(3): 205-17.
311. Noga O, Hanf G, Kunkel G. Immunological and clinical changes in allergic asthmatics following treatment with omalizumab. *Int Arch Allergy Immunol* 2003; **131**(1): 46-52.
312. Djukanović R, Wilson SJ, Kraft M, et al. Effects of treatment with anti-immunoglobulin E antibody omalizumab on airway inflammation in allergic asthma. *Am J Respir Crit Care Med* 2004; **170**(6): 583-93.
313. Prieto L, Gutiérrez V, Colás C, et al. Effect of omalizumab on adenosine 5'-monophosphate responsiveness in subjects with allergic asthma. *Int Arch Allergy Immunol* 2006; **139**(2): 122-31.

314. Brusasco V, Crimi E. Methacholine provocation test for diagnosis of allergic respiratory diseases. *Allergy* 2001; **56**(12): 1114-20.
315. Wardlaw AJ, Dunnette S, Gleich GJ, Collins JV, Kay AB. Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma. Relationship to bronchial hyperreactivity. *Am Rev Respir Dis* 1988; **137**(1): 62-9.
316. Kelly EA, Esnault S, Liu LY, et al. Mepolizumab Attenuates Airway Eosinophil Numbers, but Not Their Functional Phenotype, in Asthma. *Am J Respir Crit Care Med* 2017; **196**(11): 1385-95.
317. Haldar P, Brightling CE, Hargadon B, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med* 2009; **360**(10): 973-84.
318. Flood-Page PT, Menzies-Gow AN, Kay AB, Robinson DS. Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. *Am J Respir Crit Care Med* 2003; **167**(2): 199-204.
319. Van Den Berge M, Meijer RJ, Kerstjens HA, et al. PC(20) adenosine 5'-monophosphate is more closely associated with airway inflammation in asthma than PC(20) methacholine. *Am J Respir Crit Care Med* 2001; **163**(7): 1546-50.
320. Robinson D, Humbert M, Buhl R, et al. Revisiting Type 2-high and Type 2-low airway inflammation in asthma: current knowledge and therapeutic implications. *Clin Exp Allergy* 2017; **47**(2): 161-75.
321. Nair P, Martin JG, Cockcroft DC, et al. Airway Hyperresponsiveness in Asthma: Measurement and Clinical Relevance. *J Allergy Clin Immunol Pract* 2017; **5**(3): 649-59.e2.
322. He JQ, Hallstrand TS, Knight D, et al. A thymic stromal lymphopoietin gene variant is associated with asthma and airway hyperresponsiveness. *J Allergy Clin Immunol* 2009; **124**(2): 222-9.
323. Sverrild A, Hansen S, Hvidtfeldt M, et al. The effect of tezepelumab on airway hyperresponsiveness to mannitol in asthma (UPSTREAM). *European Respiratory Journal* 2022; **59**(1): 2101296.
324. Kaur D, Doe C, Woodman L, et al. Mast Cell-Airway Smooth Muscle Crosstalk: The Role of Thymic Stromal Lymphopoietin. *Chest* 2012; **142**(1): 76-85.
325. Marquardt DL, Gruber HE, Wasserman SI. Adenosine release from stimulated mast cells. *Proc Natl Acad Sci U S A* 1984; **81**(19): 6192-6.
326. Galdiero MR, Varricchi G, Seaf M, Marone G, Levi-Schaffer F, Marone G. Bidirectional Mast Cell-Eosinophil Interactions in Inflammatory Disorders and Cancer. *Front Med (Lausanne)* 2017; **4**: 103.
327. Chapman DG, Irvin CG. Mechanisms of airway hyper-responsiveness in asthma: the past, present and yet to come. *Clin Exp Allergy* 2015; **45**(4): 706-19.
328. Manoharan A, Lipworth BJ, Craig E, Jackson C. The potential role of direct and indirect bronchial challenge testing to identify overtreatment of community managed asthma. *Clin Exp Allergy* 2014; **44**(10): 1240-5.
329. Telenga ED, van den Berge M, Ten Hacken NH, Riemersma RA, van der Molen T, Postma DS. Small airways in asthma: their independent contribution to the severity of hyperresponsiveness. *Eur Respir J* 2013; **41**(3): 752-4.
330. Chan R KC, Lipworth B. A retrospective cohort study to evaluate the relationship of airway hyperresponsiveness to type 2 biomarkers in persistent asthma. *Arch Asthma Allergy Immunol 2021; 5: 008-031*.
331. Joos GF, O'Connor B. Indirect airway challenges. *European Respiratory Journal* 2003; **21**(6): 1050-68.

332. Lipworth BJ, Short PM, Williamson PA, Clearie KL, Fardon TC, Jackson CM. A randomized primary care trial of steroid titration against mannitol in persistent asthma: STAMINA trial. *Chest* 2012; **141**(3): 607-15.
333. Hallstrand TS, Leuppi JD, Joos G, et al. ERS technical standard on bronchial challenge testing: pathophysiology and methodology of indirect airway challenge testing. *Eur Respir J* 2018; **52**(5).
334. Calzetta L, Ritondo BL, Matera MG, Facciolo F, Rogliani P. Targeting IL-5 pathway against airway hyperresponsiveness: A comparison between benralizumab and mepolizumab. *British Journal of Pharmacology* 2020; **177**(20): 4750-65.
335. Sverrild A, Porsbjerg C, Backer V. The use of inhaled mannitol in the diagnosis and management of asthma. *Expert Opin Pharmacother* 2012; **13**(1): 115-23.
336. Currie GP, Haggart K, Brannan JD, Lee DK, Anderson SD, Lipworth BJ. Relationship between airway hyperresponsiveness to mannitol and adenosine monophosphate. *Allergy* 2003; **58**(8): 762-6.
337. Fardon TC, Pardon EJ, Hodge MR, Lipworth BJ. Comparative cutoff points for adenosine monophosphate and methacholine challenge testing. *Annals of Allergy, Asthma and Immunology* 2004; **93**(4): 365-72.
338. King GG, Bates J, Berger KI, et al. Technical standards for respiratory oscillometry. *Eur Respir J* 2020; **55**(2).
339. Juniper EF, Guyatt GH, Cox FM, Ferrie PJ, King DR. Development and validation of the Mini Asthma Quality of Life Questionnaire. *Eur Respir J* 1999; **14**(1): 32-8.
340. Portacci A, Dragonieri S, Carpagnano GE. Super-responders to biologic treatment in Type 2 high severe asthma: passing fad or a meaningful phenotype? *J Allergy Clin Immunol Pract* 2023.
341. Al-Shaikhly T, Murphy RC, Parker A, et al. Location of eosinophils in the airway wall is critical for specific features of airway hyperresponsiveness and T2 inflammation in asthma. *Eur Respir J* 2022.
342. Currie GP, Haggart K, Lee DK, et al. Effects of mediator antagonism on mannitol and adenosine monophosphate challenges. *Clin Exp Allergy* 2003; **33**(6): 783-8.
343. Altman MC, Lai Y, Nolin JD, et al. Airway epithelium-shifted mast cell infiltration regulates asthmatic inflammation via IL-33 signaling. *J Clin Invest* 2019; **129**(11): 4979-91.
344. Leckie MJ, ten Brinke A, Khan J, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000; **356**(9248): 2144-8.
345. Sehmi R, Smith SG, Kjarsgaard M, et al. Role of local eosinophilopoietic processes in the development of airway eosinophilia in prednisone-dependent severe asthma. *Clinical & Experimental Allergy* 2016; **46**(6): 793-802.
346. Sehmi R, Lim HF, Mukherjee M, et al. Benralizumab attenuates airway eosinophilia in prednisone-dependent asthma. *Journal of Allergy and Clinical Immunology* 2018; **141**(4): 1529-32.e8.
347. An J, Lee JH, Sim JH, et al. Serum Eosinophil-Derived Neurotoxin Better Reflect Asthma Control Status Than Blood Eosinophil Counts. *J Allergy Clin Immunol Pract* 2020; **8**(8): 2681-8.e1.
348. Granger V, Zerimech F, Arab J, et al. Blood eosinophil cationic protein and eosinophil-derived neurotoxin are associated with different asthma expression and evolution in adults. *Thorax* 2021.

349. Rutten B, Young S, Rhedin M, et al. Eosinophil-derived neurotoxin: A biologically and analytically attractive asthma biomarker. *PLoS One* 2021; **16**(2): e0246627.