FACTORS CONTRIBUTING TO DIFFICULT ASTHMA

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'Supposing is good, but finding out is better'

Mark Twain

Dedication

This is for my husband and best friend, Warren Tu and my daughter Sophie who fill my life with love and joy. For my mum Professor Christina Lee and dad Anthony, who have always inspired me to be my best and to strive for excellence. For my parents-in-law Quan Bu and Khanh Tu, who cared for Sophie while I was typing these pages. Finally for my supervisors Professors Mark Hew and Michael Abramson who never tired of providing mentorship, guidance and advice.

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Contents

Abstract

Asthma is a heterogeneous chronic inflammatory condition, characterised by typical symptoms of wheeze, cough, chest tightness and shortness of breath. 'Difficult asthma' has been defined as asthma that requires high doses of inhaled corticosteroids with a second preventer and/or systemic oral corticosteroids to prevent uncontrolled symptoms; or asthma that is uncontrolled despite maximal treatment. Patients with 'severe asthma' remain uncontrolled, even after alternative diagnoses are excluded and contributory factors or comorbidities are optimised. Thus patients with difficult asthma may not respond to high dose treatments, because they do not have asthma at all, because of comorbidities that can affect asthma control or due to biologically severe inflammatory disease. Specific patient factors including medication non-adherence to prescribed preventer therapies, fixed inappropriate beliefs, poor inhaler technique or chronic environmental triggers such as smoking, can also lead to asthma which is difficult and challenging to control.

With the advent of new, more expensive treatments for asthma, such as monoclonal biological therapies, it is imperative that factors contributing to difficult asthma are first accurately diagnosed and managed appropriately. While guidelines exist for the diagnosis and management of mild, moderate and severe asthma, more research is required to investigate the best way to identify and manage these factors within the difficult asthma population.

Due to the breadth of this topic, the overall aim of this thesis was to focus on just two of these factors: i) medication non-adherence, and ii) laryngeal dysfunction, comprising inducible laryngeal obstruction, also termed vocal cord dysfunction, and cough hypersensitivity, both under-recognised, but important comorbidities among patients with difficult asthma.

This thesis aimed to address gaps in knowledge relating to these two factors across five studies, using clinical data from patients attending the asthma clinic at the Alfred hospital (studies one to four) and the Alfred hospital lung function laboratory (study five).

The first two studies have been designed to examine non-adherence within a difficult asthma population. Firstly, to measure – with the use of electronic monitoring devices – the prevalence of medication non-adherence among patients who, by definition, were prescribed maximal therapy. Secondly, to apply novel descriptive metrics to medication adherence behaviour and to examine whether these metrics were a useful tool to predict associations between medication non-adherence, baseline patient characteristics, and asthma outcomes.

Validated diagnostic and treatment algorithms to identify vocal cord and laryngeal dysfunction have not yet been established. This thesis includes three studies to investigate this further: The first study evaluated patients with concomitant clinical suspicion for inducible laryngeal obstruction and asthma, and described a diagnostic strategy to elucidate these two diagnoses. The next study examined a difficult asthma population to identify clinical risk factors for the diagnosis of inducible laryngeal obstruction. The final study explored the utility of using the cough response to a bronchial provocation challenge with mannitol to identify patients at risk of laryngeal dysfunction.

In conclusion, the work in this thesis has contributed new knowledge on medication adherence and laryngeal dysfunction as factors contributing to difficult asthma.

Publications during enrolment

Publications (Included in Thesis)

1. **Lee J**, Tay TR, Radhakrishna N, Hore-Lacy F, Mackay A, Hoy R, Dabscheck E, O'Hehir R, Hew M. Non-adherence in the era of severe asthma biologics and thermoplasty. *European Respiratory Journal*; Jan 2018, 1701836; **DOI:** 10.1183/13993003.01836-2017.
2. **Lee J**, Tay TR, Paddle P, Richards AL, Pointon L, Voortman M, Abramson MJ, Hoy R, Hew M. Diagnosis of concomitant inducible laryngeal obstruction and asthma. *Clinical and Experimental Allergy* 2018; 48: 1622-1630.
3. **Lee J**, Denton E, Hoy R, Tay TR, Bondarenko J, Hore-Lacy F, Radhakrishna N, O'Hehir RE, Dabscheck El, Abramson M and M Hew. Paradoxical vocal fold motion in difficult asthma is associated with dysfunctional breathing and preserved lung function. *Journal of Allergy and Clinical Immunology in Practice* (INPRACTICE-D-19-01010). 2020;8(7):2256-2262.
4. **Lee J**, Huvanandana J, Foster JM, Reddel HK, Abramson M, Thamrin C and M Hew. Dynamics of inhaled corticosteroid use are associated with asthma attacks. *Sci Rep* 2021; **11:**14715. https://doi.org/10.1038/s41598-021-94219-z
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Rhinitis: The TAISAR study. *Journal of Allergy and Clinical Immunology*. Available online 10 November 2021. *Article in Press.*

Oral Presentations (National/International Conferences)

1. **Lee J**, Tay TR, Hoy R, Paddle P, Richards A, Pointon L, Voortman M and M Hew. Middle airway symptoms and suspected vocal cord dysfunction an analysis of patients referred for systematic multidisciplinary assessment. TSANZ Victoria Branch Annual Scientific Meeting. November 2016, Melbourne, Australia.
2. **Lee J**, Tay TR, Radhakrishna N, Hore-Lacy F, Hoy R, Dabscheck E, O'Hehir R and Hew M. Medication adherence in a difficult asthma population**.** Oral presentation at TSANZ conference March 2017, Canberra Australia.
3. **Lee J**, Huvanandana J, Foster JM, Reddel HK, Abramson M, Thamrin C and M Hew. Entropy analysis of inhaled preventer use predicts asthma attacks. Oral presentation at TSANZ virtual conference May 2021, Australia.

Poster Presentations (National/International Conferences)

1. **Lee J**, Tay TR, Radhakrishna N, Hore-Lacy F, Hoy R, Dabscheck E, O'Hehir R and Hew M Medication adherence in a difficult asthma population**.** Internal Medicine Journal. 2016;(46)(S4):17. Poster presented at ASCIA conference 2016, Gold Coast, Australia.
2. **Lee J**, Tay TR, Radhakrishna N, Hore-Lacy F, Smith C, Hoy R, Dabscheck E, O'Hehir R and Hew M. Risk factors for vocal cord dysfunction in a difficult asthma population. Internal Medicine Journal. 2016;(46)(S4):17. Poster presented at ASCIA conference 2016, Gold Coast, Australia.

3. **Lee J,** Kronborg C and Hew M. Thunderstorm asthma: Patient outcomes and clinical review. Poster presented at the European Respiratory Society International Congress, September 2017, Milan, Italy.

Prizes and Awards During Enrolment

1. The Australasian Society of Clinical Immunology and Allergy (ASCIA) and National Asthma Council Australia (NAC) Asthma Research Award September 2016, Gold Coast, Australia.
2. Thoracic Society of Australian and New Zealand (TSANZ) and Australia and New Zealand Society of Respiratory Science (ANZSRS) Victorian Branch Annual Scientific Meeting Award, November 2016, Melbourne, Australia.
3. Thoracic Society of Australian and New Zealand (TSANZ) and Australia and New Zealand Society of Respiratory Science (ANZSRS) National Conference SIG Oral Award: Asthma and Allergy March 2017, Canberra Australia.
6. School of Public Health and Preventive Medicine Excellence Awards. 2018 Best Paper Award for: **Lee J**, Tay TR, Radhakrishna N, Hore-Lacy F, Mackay A, Hoy R, Dabscheck E, O'Hehir R, Hew M. Non-adherence in the era of severe asthma biologics and thermoplasty. *European Respiratory Journal*; Jan 2018, 1701836; **DOI:** 10.1183/13993003.01836-2017.
7. The best of the European Respiratory Journal abstracts presentation for: **Lee J**, Tay TR, Radhakrishna N, Hore-Lacy F, Mackay A, Hoy R, Dabscheck E, O'Hehir R, Hew M. Non-adherence in the era of severe asthma biologics and thermoplasty. *European Respiratory Journal*; Jan 2018, 1701836; **DOI:** 10.1183/13993003.01836-2017. September 2018, Paris, France.

8. Featured in “Journal Watch”, by the New England Journal of Medicine for: **Lee J**, Denton E, Hoy R, Tay TR, Bondarenko J, Hore-Lacy F, Radhakrishna N, O’Hehir RE, Dabscheck El, Abramson M and M Hew. Paradoxical vocal fold motion in difficult asthma is associated with dysfunctional breathing and preserved lung function. Journal of Allergy and Clinical Immunology in Practice (INPRACTICE-D-19-01010). 2020; 8(7):2256-2262. July 2020.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Signature:

Print Name: Joy Lee

Date: 20th January 2022

Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes five original papers published in peer reviewed journals. The core theme of the thesis is factors contributing to difficult asthma. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the School of Public Health and Preventive Medicine, under the supervision of Professors Mark Hew and Michael Abramson.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of chapters three to six, my contribution to the work involved the following:

Thesis Chapter	**Publication Title**	**Status**	**Nature and % of student contribution**	**Co-author name(s) Nature and % of Co-author's contribution***	**Co-author Monash student Y/N**
3	Non-adherence in the era of severe asthma biologics and thermoplasty.	Published	60% Study conception, study design, acquisition of data, data	10% Tay TR – data acquisition, analysis, manuscript preparation 5% Radhakrishna N - data acquisition,	N

			analysis and interpretation, manuscript preparation	analysis, manuscript preparation 3% Hore-Lacy F - data acquisition, manuscript preparation 3% Mackay A - data acquisition, manuscript preparation 3% Hoy R, data acquisition, manuscript preparation 3% Dabscheck E - data acquisition, manuscript preparation 3% O'Hehir R - data acquisition, manuscript preparation 10% Hew M – data acquisition, data analysis, study concept, manuscript preparation	
4	Entropy analysis of inhaled preventer use predicts asthma attacks	Published	55% Study conception, study design, acquisition of data, data analysis, and interpretation, manuscript preparation	10% Huvanandana J – data analysis and interpretation, manuscript preparation 5% Foster JM – study conception, design, manuscript preparation 5% Reddel HK - data interpretation and manuscript preparations 5% Abramson M – data analysis, interpretation, manuscript preparation 10% Thamrin C – study conception, study design, data analysis and interpretation,	N

				manuscript preparation 10% Hew M. study conception, study design, data interpretation, manuscript preparation	
5	Diagnosis of concomitant inducible laryngeal obstruction and asthma.	Published	57% Study conception, study design, acquisition of data, data analysis and interpretation, manuscript preparation	10% Tay TR – data acquisition, data analysis and interpretation, manuscript revision 3% Paddle P – data acquisition, manuscript revision 3% Richards AL - data acquisition, manuscript revision, 3% Pointon L - data acquisition, manuscript revision 3% Voortman M - data acquisition, manuscript revision 5% Abramson MJ data analysis and interpretation, manuscript revision 8% Hoy R – data acquisition, manuscript revision 8% Hew M – data analysis and interpretation, manuscript revision	N
6	Paradoxical vocal fold motion in difficult asthma is associated with dysfunctional breathing and preserved lung function	Published	51% Study conception, study design, acquisition of data, data analysis and interpretation, manuscript preparation	8% Denton E – data acquisition, manuscript revision 5% Hoy R - data acquisition, manuscript revision 5% Tay TR - data acquisition, manuscript revision 3% Bondarenko J - data acquisition, manuscript revision 3% Hore-Lacy F - data acquisition,	Y

				manuscript revision 3% Radhakrishna N - data acquisition, manuscript revision 3% O'Hehir RE - data acquisition, manuscript revision 3% Dabscheck El - data acquisition, manuscript revision 8% Abramson M - data interpretation, manuscript revision 8% M Hew – data analysis and interpretation, manuscript revision	
7	Laryngeal hypersensitivity in patients undergoing bronchoprovocation challenge with mannitol	Published	65% Data acquisition, data analysis, and interpretation, manuscript preparation	8% Tay TR – study concept and design, data acquisition, manuscript preparation 3% Borg BM – data acquisition, manuscript preparation 3% Sherriff N – data acquisition 3% Vertigan A – data interpretation and manuscript preparation 8% Abramson M – data analysis and interpretation, manuscript preparation 10% Hew - study concept, design, data interpretation, manuscript preparation	N

I have renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student name: Dr Joy Lee

Student signature: **Date: 20 January 2022**

I hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author, I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor name: Professor Mark Hew

Main Supervisor signature: **Date:**

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This thesis would not have been possible without the unwavering and patient support of my two supervisors, Professor Mark Hew and Professor Michael Abramson. I truly appreciate the time that you have both set aside for me to provide the guidance and encouragement to help me achieve this goal. I have benefitted so much from your experience and advice, you have both shaped the academic and clinical spheres of my career.

I wish to acknowledge my colleagues within the Asthma, Allergy and Immunology department at Alfred health – including nursing staff, physicians, allied health team members and respiratory scientists – the published works in this thesis would not have been possible without you, thank you.

I am also grateful for the financial support to pursue postgraduate research provided to me by the Australian Government Research Training Program Scholarship.

For my family, Warren and Sophie, who are my world, I love you both.

And finally, I wish to acknowledge and thank all of the patients who contributed data to this research, and for all future asthma patients, I hope that this research can help to make your lives a little bit better.

List of Figures

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Chapter One: Introduction

1.1 Aims of the Thesis

The overall aim of this thesis was to investigate patients with difficult asthma, focusing on identifying the contributing factors of medication adherence and laryngeal hypersensitivity, so that strategies could be undertaken to improve patient management and outcomes.

1.1 This thesis evaluated these factors contributing to difficult asthma via the following five research aims:

1. To quantify the prevalence of medication non-adherence in a difficult asthma population.
    a. To compare the accuracy of identification of non-adherence among self-report, health carer assessment and electronic monitoring devices.
2. To use inhaler data from electronic monitoring devices to develop metrics, particularly entropy, to describe medication adherence behaviour in difficult asthma.
    a. To develop metrics to predict patient characteristics at risk of medication non-adherence.
    b. To examine the association between abnormal medication adherence behaviours with adverse asthma-related clinical outcomes.
3. To evaluate patients with concomitant clinical suspicion for vocal cord dysfunction (inducible laryngeal obstruction) and asthma based on a systematic evaluation process to differentiate the two diagnoses.
4. To identify baseline clinical factors that were associated with the comorbidity of vocal cord dysfunction among patients with difficult asthma.

5. To describe the utility of the cough response to bronchial provocation testing in identification of patients with laryngeal hypersensitivity.

1.1 These aims relate to the following hypotheses:

1. Despite being referred by specialists, medication non-adherence among patients with difficult asthma is highly prevalent.

2. Medication non-adherence is not able to be identified accurately by subjective measurements.

3. Data metrics and entropy measurement may be used to predict characteristics of patients at risk of medication non-adherence.

4. Poor medication adherence as measured by entropy (a metric to describe the chaotic irregularity of inhaler use) is related to adverse asthma outcomes and comorbidities.

5. Clinical factors can be identified to predict the risk of laryngeal dysfunction, particularly VCD, among patients with difficult asthma.

7. Cough frequency during bronchial provocation testing can detect laryngeal dysfunction.

These aims are addressed in five published works, which are included as the major components of this thesis.

1.2 Conceptual Framework and Thesis Structure

To understand the relationship between severe and difficult asthma, the Hew and Chung model(1) detailing the interaction of different factors contributing to difficult asthma has been adopted as a conceptual framework for this thesis.

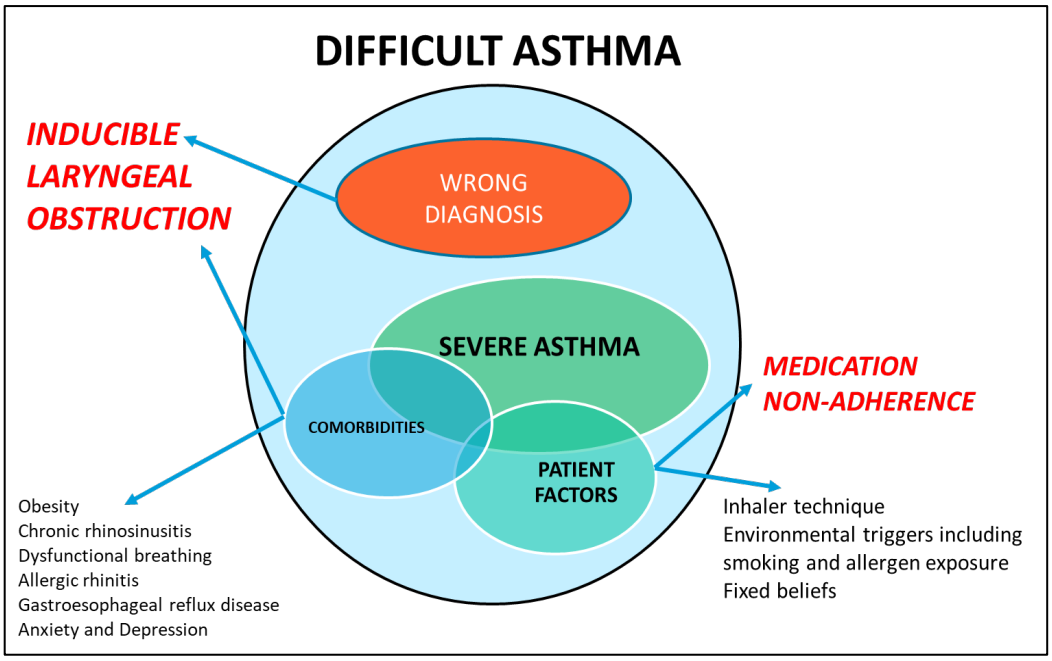

Figure 1. Theoretical Framework for the relationship between difficult asthma, severe asthma, patient factors and comorbidities. Adapted from: Hew M, Chung KF. Corticosteroid insensitivity in severe asthma: significance, mechanisms and aetiology. Intern Med J 2010: 40(5): 323-334. Used with permission, License number: 4471681230435.

Intrinsic disease severity represents only a subset of patients with difficult asthma. This model highlights the importance of confirming the diagnosis of asthma, and conducting a thorough assessment for multiple comorbidities and patient factors before a diagnosis of severe asthma is made.(2-4)

Given the many possible factors that could contribute to difficult-to-treat asthma, a decision was made to focus this thesis on two risk factors, including medication non-adherence and laryngeal dysfunction (including ILO/VCD and cough hypersensitivity).

Limiting the thesis to these two major themes allowed a more comprehensive and deeper evaluation of these topics where more specific and detailed research questions could be posed. These two factors were chosen in particular due to the significant knowledge gaps that were identified in a literature review on a variety of risk factors for difficult-to-treat asthma (section 1.3 of this Chapter). Our centre was also uniquely placed to evaluate these two factors given our early adaptation of medication adherence technology with electronic monitoring devices, and our unique multidisciplinary service set up to evaluate difficult-to-treat asthma with comorbid VCD. Medication non-adherence and inducible laryngeal obstruction/VCD were also highlighted as key "treatable traits" on which to focus research efforts at a recent Centre of Research Excellence Annual research meeting of experts (see further detail on this in Chapter 8, Section 8.4.3). Other associated comorbidities and risk factors were outside the scope of this thesis.

Chapter One is a general introduction and literature review which highlights the knowledge gaps that exist with respect to the thesis aims. Chapter Two outlines the general methods used within this thesis. A summary of the thesis structure, including the studies that address the thesis aims within two sections, is conveyed below in Figure One.

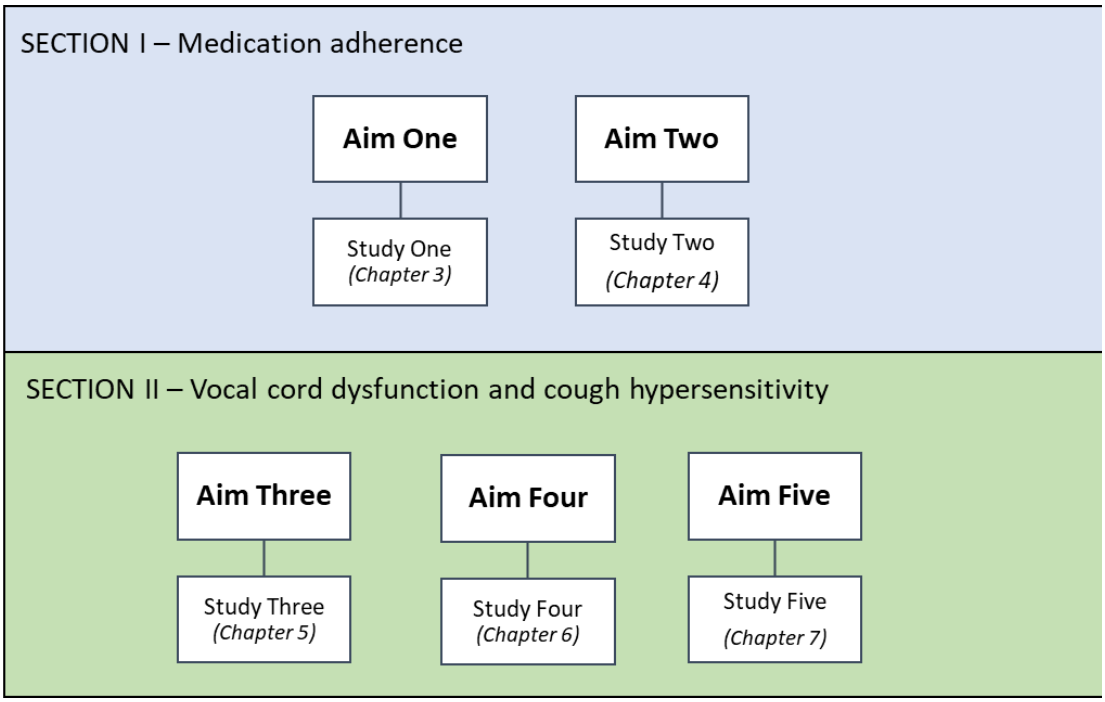

Figure 2: A summary of the thesis structure

Section I of this thesis addresses medication non-adherence as a patient factor underlying difficult asthma. The first thesis aim, to quantify the prevalence of non-adherence to preventer inhalers within a difficult asthma patient population was explored in study one (*Chapter Three*), and the second thesis aim, to develop adherence metrics to describe medication adherence, in study two (*Chapter Four).* Section II of this thesis examines the middle airway – comprising the larynx, vocal cords and cough reflex as contributing comorbidities or misdiagnoses within difficult asthma. Section II comprises studies that evaluated aims three (to evaluate patients with potential co-diagnoses of both asthma and vocal cord dysfunction and to determine differentiating diagnostic features) and four (to identify clinical features that predict the presence of VCD as a comorbidity in difficult asthma) within *Chapters Five* and *Six* respectively. *Chapter Seven,* the last chapter of Section II, contains the final study of this thesis, which examines the cough response and laryngeal dysfunction among patients undergoing bronchial provocation challenge testing with

mannitol. *Chapter eight* presents the final discussion and concluding remarks including suggestions for future research directions and implications for clinical practice.

1.3 Narrative Literature Review

1.3.1 Introduction and Methods

This narrative literature review is a pragmatic and targeted overview on factors contributing to difficult-to-treat asthma to place the findings of this thesis into context. It begins with the definitions used for severe, uncontrolled and difficult-to-treat asthma, followed by the prevalence and burden of this condition, particularly within an Australian context. The third section comprises a review of the current literature on the risk factors for difficult-to-treat asthma, followed by the knowledge and research gaps that were addressed by this thesis for relevant risk factors. A narrative review is a useful methodology to appraise previous studies and highlight the rationale for future research, however its main limitation would be subjective study selection which may lead to a selection bias in the literature considered.

1.3.2 Definitions and Distinction Between Uncontrolled, Difficult-to-treat and Severe Asthma

Asthma is a heterogeneous, chronic lung disease characterised by underlying bronchial airway inflammation and hyperreactivity.

The European Respiratory Society (ERS) Taskforce on Severe Asthma defines uncontrolled asthma as one or both of: asthma with poor symptom control and or frequent exacerbations requiring two or more courses of oral corticosteroids in the preceding year, or at least one serious exacerbation requiring hospitalisation, intensive care unit (ICU) admission or mechanical ventilation.(5) The Global Initiative for Asthma (GINA) defines difficult-to-treat asthma as asthma that remains uncontrolled despite moderate to high dose preventer therapy with inhaled corticosteroids and a second controller (long acting

bronchodilators) and/or oral corticosteroids, or asthma that requires this amount of treatment to maintain symptom control and reduce asthma exacerbations.(6) Severe asthma can be defined as asthma that is uncontrolled despite adherence to maximally optimised therapy and treatment of contributory factors.(5) Severe asthma may thus be considered a subset of difficult-to-treat asthma and a retrospective diagnosis.(6) It is important to differentiate severe asthma from difficult asthma due to the differing approaches to therapy, with standard treatments optimised and contributory factors addressed before multiple new and often more expensive treatment options for severe asthma are pursued.

1.33 Prevalence and Burden of Asthma and Difficult-to-treat Asthma

Asthma is a serious global health issue, and its prevalence is particularly high within high income countries, including Australia.(7) Despite advances in asthma management strategies, including preventer inhaled corticosteroids and monoclonal biologics, asthma continues to place a significant burden on patients, their families and national healthcare systems. Globally, in 2019, asthma affected an estimated 262 million people and caused 461,000 deaths. In 2016, asthma contributed globally to 23.7million Disability Adjusted Life Years (DALYs).(8) In Australia, 2.7 million (11% of the population) self-reported that they had asthma in the 2017-2018 Australian Bureau of Statistics National Health Survey. (9) Asthma-related deaths in Australia remain among the highest in the world, and in 2019, there were 421 asthma-related deaths reported in Australia.(10) In Australia, asthma contributed 34% of the total burden of disease due to respiratory conditions and 117,000 DALYs(11).

In 2016, the costs of asthma on the Australian health system totalled an estimated A$770 million, 19% of all disease expenditure for any respiratory condition. Of this total expenditure, $205million was in hospital costs (27%), and $383 million for pharmaceuticals (50%). However when other indirect costs including loss of productivity and loss of healthy life were taken into account, this amount was thought to be much higher at an estimated $28billion or a cost of $11,470 per person with asthma.(12)

In a Danish study of over 1000 patients with asthma, 17% were classified as having difficult-to-control asthma. Of the patients with difficult asthma, only 12% had truly severe asthma.(13). Over half had difficult asthma due to medication non-adherence and inhaler technique issues. Unmanaged comorbidities were also identified in 67%. In a Dutch adult survey extrapolated to the Netherlands population, 17.4% of patients with asthma were classified as having difficult asthma, and only 3.6% were classified with truly severe asthma after assessment of medication adherence and inhaler technique.(14)

Closer to home, in a cross-sectional web-based survey of 2686 Australian adults with asthma, 19.7% had uncontrolled symptoms despite regular inhaled preventer use and 10% of asthma patients had seen a specialist.(15) In our health service at the Alfred health in Melbourne, Australia, a general asthma clinic was surveyed to identify patients with asthma who were assessed by their specialist to have difficult-to-control asthma. This survey found that about 10% of asthma patients were considered “difficult” by respiratory specialists.(16) Poor control of asthma symptoms (62%), frequent exacerbations (44%), poor lung function (42%) , patient factors (29%) and diagnostic dilemmas (26%) were common reasons cited as to why the patient’s asthma was considered to be difficult to control. Over three quarters (80%) of these patients assessed also had severe asthma by ERS/ATS definitions.(16)

While difficult asthma may affect only a small proportion of all patients with asthma, these patients often come with significant challenges including greater treatment burdens, morbidities and greater associated healthcare costs.(17) In an economic analysis from the British Thoracic Society Difficult asthma registry, annual mean treatment costs among severe refractory asthma were between £2912 and £4217, including costs of general practitioners, emergency department and hospital visits. (18) A Canadian study estimated that patients with severe uncontrolled asthma were responsible for use of 94% of asthma-related health care resources.(19)

1.3.4 Risk Factors for Difficult-to-treat Asthma

Among patients with difficult asthma, poor asthma control is more commonly due to factors other than intrinsically severe asthma biology, including: incorrect diagnosis, poor medication adherence, incorrect inhaler technique, triggers with ongoing environmental exposure, and/or exacerbating comorbidities. These factors have also been termed 'treatable traits' as potentially modifiable characteristics that can be specifically treated to improve disease outcomes.(20, 21) The focus of this thesis and review is specifically on incorrect diagnosis, poor medication adherence and exacerbating comorbidities, in particular, laryngeal hypersensitivity and vocal cord dysfunction.

Incorrect diagnosis

Objective demonstration of variable airflow obstruction is required to confirm the diagnosis of asthma, but is often not undertaken in primary or even secondary care.(22) Airflow obstruction is seen during spirometry when the forced expiratory volume in one second over the forced vital capacity ratio is less than 70% or below the lower limit of normal. Variable airflow obstruction can be demonstrated by bronchodilator

responsiveness in FEV_1 and/or FVC values following bronchodilator administration which are greater than 12% and 200mL compared to baseline during testing considered a “significant” change.(23) Other methods of demonstrating variable airflow obstruction include measurements of peak expiratory flow rates over a period of two to four weeks demonstrating more than 20% variability during this time and bronchial provocation challenge testing.(24) Due to the intermittent nature of asthma, spirometry may be normal, therefore necessitating the use of these other methods.

Bronchial challenge agents may act directly on airway smooth muscle receptors (for example, histamine and methacholine) or indirectly via inflammatory mediators, such as mannitol, exercise or allergen challenges.(25) In a study of 123 patients with asthma in the community who underwent bronchial challenge testing to both methacholine and mannitol, 30% were non-responsive to both challenges, and were thought to be either misdiagnosed or overtreated.(26) Similarly, in a Canadian study of 613 patients who had been diagnosed with asthma by a physician, after a series of objective testing including spirometry and bronchial challenge testing, 33% had asthma ruled out as a diagnosis. Alternative diagnoses included gastroesophageal reflux disease, anxiety or hyperventilation, chronic rhinitis, obesity or deconditioning, eosinophilic bronchitis, ischaemic heart disease, chronic obstructive pulmonary disease (COPD) and post-viral cough.(27)

In an Italian study investigating the prevalence of misdiagnosed asthma among patients referred to an allergy clinic, 51.2% had a negative bronchial challenge test.(28) Other studies which utilised a systematic assessment of difficult-to-treat asthma also found a proportion of patients did not have asthma even in patients who had previously been seen by specialists.(2, 29) In an Australian study of 90 difficult-to-treat asthma patients

who had been seen by respiratory specialists, 5 (5.5%) had asthma excluded as a diagnosis, with other diagnoses including COPD, vocal cord dysfunction and dysfunctional breathing being alternative explanations for the patients' symptoms.

Knowledge gaps

Indirect bronchial provocation challenge testing with mannitol is available in a ready-to-use kit, is relatively well tolerated and has a high diagnostic specificity for asthma.(30, 31) It is therefore a useful adjunct for the objective demonstration of variable airflow obstruction to confirm an asthma diagnosis.(30) However, it was not known whether it may also be used to identify alternative causes for patient symptoms, if it proves negative for bronchial hyperreactivity. Adding other components to this investigation such as comorbidity questionnaires and cough counting measurements may be helpful to diagnose laryngeal dysfunction as an alternative explanation for the patient's symptoms, including vocal cord dysfunction and chronic cough hypersensitivity, and are investigated in *Chapter Seven*.

Vocal cord dysfunction, also termed indirect laryngeal obstruction has been previously identified as a common differential diagnosis to asthma among patients presenting with difficult-to-treat asthma and is recommended in diagnostic guidelines to be considered as a differential diagnosis (16, 32-35). However, there was no recognised gold standard for a systematic assessment to distinguish between the two diagnoses. Given the treatment for the two conditions is vastly different, this is an important knowledge gap to address, and is investigated in *Chapter Five* of this thesis and elaborated further in this review, under *Comorbidities*.

Inadequate Medication Adherence

Medication non-adherence is well described among patients with any chronic disease. Fifteen years ago, the World Health Organisation identified asthma as one of nine globally important chronic conditions for which medication adherence must be optimised.(36) Inhaled corticosteroids (ICS) are an effective treatment for the majority of patients with asthma and significantly reduce the risk of requiring systemic corticosteroids, severe asthma exacerbations and death.(37) However, despite this, many patients with asthma of all severities do not take their inhalers as prescribed. There has been no change to overall rates of adherence among patients with asthma over the last three decades.(38-42) Progress will continue to be slow, until there is improvement in the measurement of adherence behaviour.(43)

Adherence with asthma medications in adults may range from 30% in the "real world" (40) to 70% (44) or more when patients have been aware of medication monitoring in a clinical trial setting. High rates of non-adherence among patients with difficult asthma have been previously demonstrated by monitoring prescription refills(2, 45). In a study by Gamble and colleagues of 182 patients with difficult asthma, 88% admitted to poor adherence to inhaled steroids and 35% filled 50% or less of their prescriptions.(46) Non-adherence was more frequently seen in women and was associated with higher rates of exacerbations requiring hospitalisation or nebulised bronchodilators. In a case series, 50% of patients prescribed oral steroids were found to be non-adherent when they were assessed by serum prednisolone and cortisol measurements.(2) In another study from Leicester, 65.2% of patients (n=115) filled less than 80% of their prescriptions for inhaled corticosteroids.(45)

In 2013, only 17% of Australians using any ICS containing medication had enough dispensed to have taken it for at least half of the time that it was prescribed.(47) An Australian retrospective cohort study of Pharmaceutical Benefits Scheme (PBS) asthma inhaler dispensing data in 2018 found that among patients with asthma who had been dispensed

potentially toxic doses of oral corticosteroids (cumulatively over 1000mg prednisolone in the previous 12 months), 50% of those dispensed high dose ICS/LABA inhaler had fewer than 50% of their scripts dispensed.(48) Similarly, in a trajectory analysis of adherence to ICS/LABA from Australian national dispensing data, over 80% of GINA step 5 treatments (oral corticosteroids, long acting muscarinic agents or biologics) were commenced in poorly adherent patients.(49)

Non-adherence has been associated with poorer lung function and increased risk of asthma related morbidity including exacerbations requiring mechanical ventilation and asthma-related mortality.(50, 51) However many methods of adherence detection are flawed, for example in the Australian setting, the method of adherence detection by prescription filling may be inaccurate as patients may obtain their preventers from different prescribers, and dispensed from multiple pharmacies. This method of adherence detection also does not identify "dose-dumping" or stockpiling. In addition, serum prednisolone levels may not be available at all labs or could also represent poor absorption. Patient self-report or diaries can be unreliable due to poor recall and bias(52) and health care professional assessment of non-adherence may also be inaccurate. Non-adherence may also be more objectively identified by weighing inhaler devices,(53) high sputum eosinophils,(45) fractional exhaled nitric oxide (54-56) or by electronic monitoring device.(57-59)

There may be many reasons for poor adherence to medication, which can be intentional and/or unintentional.(60) For example, patients may believe their treatment is unnecessary or dangerous with fear of adverse effects, they may be embarrassed to use their inhalers or be influenced by family and friends' opinions.(61) Unintentional causes of non-adherence include forgetfulness, poor inhaler technique (62), difficulty affording prescriptions or a poor understanding of the treatment regimen prescribed.(63) Patients may prefer to use short acting bronchodilator relievers rather than corticosteroid-based

preventers. In an Australian study of 100 patients with asthma, 33% reported only taking their preventer when they had symptoms and 19% did not have a daily medication routine. Nearly half (48%) of patients disliked the idea of using an inhaled steroid, 24% had difficulty motivating themselves to take their medication and a third of patients reported side effects of ICS. Factors that were significantly associated with poor medication adherence included patients perceiving the inhaler was unnecessary, safety concerns, non-acceptance of asthma chronicity or medication effectiveness, poor motivation or lack of routine, difficulty with use of the inhaler and poor satisfaction with asthma management.(61)

Knowledge gaps

There are no agreed guidelines on how inhaler non-adherence is best addressed, measured and confirmed, especially prior to progressing asthma treatments to more expensive, or invasive, targeted options such as monoclonal biological therapies and bronchial thermoplasty.(64) While severe asthma is defined as asthma that fails to respond to high dose inhaled therapy,(6) it is not clear how adherence to this high dose therapy should be assessed. The development of strategies and interventions to allow effective identification and management of medication non-adherence among patients with difficult asthma have been highlighted as clinical priorities by international taskforces.(65) It is estimated that with addressing of adherence and inhaler technique, that the prevalence of truly refractory severe asthma would reduce to less than 5% of all asthmatics.(14)

While the true prevalence of non-adherence to inhaled therapies has been described in general among patients with asthma in Australia(47, 48) and in primary care (15), it has not been clearly described in a difficult asthma population, particularly among those that may be eligible for targeted treatments. This research question is addressed in *Chapter Three* of this thesis.

In addition, measurement of adherence by time-averaged metrics such as mean adherence (the total doses taken divided by the total doses described) fail to capture the effects of timing of doses or specific variations in medication behaviours and have not been consistently linked with asthma outcomes.(66) A recently published systematic review and meta-analysis of adherence to inhaled ICS in early adulthood, stated that more reliable and objective measures of adherence are needed to precisely characterise adherence.(67) This knowledge gap is addressed in *Chapter Four* of this thesis.

Poor inhaler technique

Even if patients are compliant with an inhaler regime as prescribed, they may still fail to respond to treatment due to failure of delivery of ICS to the airways. In this regard, drug delivery by inhalation is significantly more difficult when compared to drug delivery by other routes such as orally or intravenously.(68) Patients may be unable to use their inhaler effectively, or know how to use the inhaler, but choose to use it non-effectively, for example not using a spacer due to inconvenience. Inhaler misuse is common among patients with asthma.(69) Different techniques are required for different types of inhalers, adding further complexity for patients on multiple inhalers.(70)

In a study of chronic obstructive pulmonary disease (COPD) patients by Sulaiman and colleagues using an acoustic remote monitoring device attached to patients' inhalers, the mean number of doses taken from the inhaler was 59.8%. However, once errors in inhaler technique were included, the overall actual adherence rate was only 22.6%.(71) In a longitudinal study by the same authors of 123 patients with asthma and COPD, only 20% of patients used their inhaler in the correct manner. Common errors included inadequate inspiratory flow (27%), drug priming without inhalation (19%), exhaling into the inhaler (18%) and multiple inhalations (25%).(72)

Other studies have demonstrated that some types of inhaler devices are more prone to errors in use than others. In an Italian study the rate of errors for the Turbuhaler™ was 44% compared to 12% for metered dose inhalers.(73) Inhaler misuse was associated with older age, less education and lack of instruction by health care professionals, and was also associated with an increased risk of exacerbations requiring hospitalisation, oral corticosteroid use and poor disease control.(73) Similarly, the CRITIKAL study assessed inhaler use in over 3000 asthma patients and identified that users of dry powder devices commonly made insufficient inspiratory efforts. This was associated with uncontrolled asthma and increased asthma exacerbation, while errors in metered dose inhaler use were associated with poorer asthma control, but not associated with risk of exacerbation.(74)

Triggers

Environmental exposures and physiological factors can trigger asthma and increase risk of exacerbations, with most evidence from population-based cross-sectional or cohort studies.(75) Triggers can be classed as avoidable or unavoidable. Common avoidable triggers include cigarette smoke,(76) allergen exposures in atopic individuals (including thunderstorms in grass pollen seasons),(77-79) cold air, wood smoke,(80) household aerosols and strong odours, moulds, occupational exposures,(81) medications such as non-steroidal anti-inflammatory drugs, aspirin and beta-blockers, food chemicals and air pollutants.(82, 83) In a large longitudinal analysis of six European cohorts, asthma incidence was weakly associated with nitrogen dioxide and nitrogen oxide levels suggesting a deleterious effect of ambient air pollution on the incidence of asthma in those countries.(84) However, a recent workshop report from the American Thoracic society on outdoor air pollution and new-onset airways disease concluded that while there is evidence

supporting a relationship between air pollution and childhood asthma, the evidence in adult asthma was insufficient with further research needed.(85)

Knowledge gaps

These triggers may also trigger other conditions such as vocal cord dysfunction, a focus of this thesis. Identifying differences between types of triggers for asthma compared to vocal cord dysfunction may help in differentiating the diagnoses and, if both co-exist, identify which condition is contributing the most to the patient's burden of symptoms. A previous conference abstract reported 202 patients with VCD and identified that talking, shouting, swallowing and the scent of vinegar were reported to be more common among patients with VCD, compared to pollen exposure and damp air being common among patients with asthma.(86) However further studies are required to confirm these findings, and this knowledge gap is addressed in *Chapter Five* of this thesis.

Comorbidities

A comorbidity is the co-occurrence of another disease or disorder in the same patient. In asthma, comorbidities can also be considered as "treatable traits" and part of the patient's asthma phenotype.(20) Comorbidities in asthma may be classified as pulmonary (including conditions affecting the upper, middle and lower airways) or extrapulmonary (such as anxiety and depression, dysfunctional breathing, obesity, gastroesophageal reflux disease, osteoporosis, cardiovascular and metabolic disease).(87) Common upper airway comorbidities include: allergic rhinitis, chronic rhinosinusitis (with or without nasal polyposis) and obstructive sleep apnoea. Common middle airway comorbidities include vocal cord dysfunction(35) and chronic cough hypersensitivity.(88, 89) Common lower

airway comorbidities include: bronchiectasis and chronic obstructive pulmonary disease.(90)

Comorbidities are even more highly prevalent among patients with severe and difficult-to-treat asthma, compared to less severe asthma, and may be underdiagnosed. In a Dutch study of over 900 severe asthma patients, 75% had at least one comorbidity.(91) Several comorbidities may also co-exist in the one patient. In a study of patients with difficult-to-treat asthma, the median number of comorbidities per patient was three.(16) Specific questionnaires can improve diagnostic identification of comorbidities. In a study by Radhakrishna et al, the use of validated questionnaires significantly heightened detection of comorbidities when compared to physician assessment alone.(92)

Comorbidities can be misdiagnosed as asthma,(93) as well as impact asthma control and quality of life,(94) increase the risk of asthma exacerbations(20, 95) and affect effectiveness of asthma treatments.(1, 87) A multidisciplinary and systematic approach to the diagnosis and management of comorbidities is essential and improves asthma outcomes.(4, 96)

Vocal cord dysfunction or Inducible laryngeal obstruction is an important comorbidity in difficult-to-treat asthma

The terms 'inducible laryngeal obstruction' (ILO) or 'vocal cord dysfunction' (VCD), respectively refer to inappropriate adduction or narrowing of the larynx and vocal cords. The term ILO is thought to be more technically correct, as the level of laryngeal obstruction can occur at both glottic and supraglottic levels; (97) however VCD is probably the term used more commonly by clinicians.(98, 99) The visualisation of paradoxical vocal cord adduction during a symptomatic event is the gold standard for diagnosis. The underlying pathophysiology is thought to be related to hypersensitivity of the larynx,(100) which may be triggered by relatively minor irritants such as odours or other environmental

irritants,(101, 102) emotional stress, exercise(103, 104), laryngopharyngeal reflux(105) or post-nasal drip.(3)

VCD is therefore part of a larger group of disorders caused by dysfunction of the larynx, including chronic cough, muscle tension dysphonia and *globus pharyngeus*.(106) Common presenting symptoms such as dyspnoea, chest tightness, wheeze and frequent coughing significantly overlap with asthma. Asthma and VCD can also coexist, and this occurs in as many as 30-50% of difficult asthma patients in some case series (34, 92). In a recent cross-sectional observational study of 97 participants comparing patients with severe asthma and laryngeal dysfunction, 87% of patients with severe asthma had laryngeal dysfunction which affected respiration, phonation or both.(107) This creates further challenges for accurate classification of the two conditions.(34)

Patients with VCD may be over-represented among patients with difficult asthma due to misattribution of symptoms and a subsequent failure to respond to standard asthma therapies. Furthermore, patients with this comorbidity have inferior disease outcomes, with increased symptoms, more frequent exacerbations and poorer quality of life.(95) In an Australian registry of 434 severe asthma patients, the presence of vocal cord dysfunction as a comorbid treatable trait was one of the strongest predictors of exacerbation risk with an odds ratio of 1.51 (95%CI 1.22-1.88).(20) VCD is often under-recognised by respiratory physicians(92) and incompletely understood with subsequent delays in diagnosis.(108) In a retrospective analysis of 292 patients with either VCD, asthma or coexisting VCD and asthma, 42.4% of those with VCD had been previously misdiagnosed as having asthma, with an average period of misdiagnosis of nine years.(109) Validated diagnostic and treatment algorithms have not yet been established.

Several questionnaires have been developed to improve diagnosis, but have not been comprehensively validated in other centres. The Pittsburgh Index comprises four symptoms: change in voice, absence of wheeze, throat tightness, and symptoms triggered by odours. When positive, it has a reported sensitivity of 83% and specificity of 95% for the diagnosis of VCD, however this study excluded patients with coexisting VCD and asthma.(110)

The VCD-Q is a 12-point questionnaire which was developed as a symptom monitoring tool, with scores over 50 more suggestive of VCD. While healthy controls tended towards low scores under 15, patients with asthma without VCD could also score highly, giving it poor discriminating ability between asthma and VCD.(111) The dyspnoea index was developed as a 10 point Likert scale with a focus on upper airway symptoms.(112). It is more useful to assess symptom severity and assess response to treatment rather than a diagnostic tool, as it is not specific for VCD.

The Newcastle laryngeal hypersensitivity questionnaire was developed following prospective evaluation of patients with laryngeal dysfunction in comparison to a healthy control group. It is helpful to identify patients with laryngeal dysfunction –which also includes chronic cough, *globus pharyngeus* and muscle tension dysphonia in addition to VCD.(113)

A study of 123 patients with abnormal inspiratory curves on flow volume loops found a specific aetiology for this abnormality in 52% of patients, with vocal cord dysfunction being the most frequent diagnosis.(114) It has also been demonstrated that maximum mid inspiratory flow (MIF50) best reflects changes in mid inspiratory glottic area.(115, 116) However subsequent studies have shown that a flattened inspiratory loop might have limited diagnostic value even when symptomatic, with reported sensitivity of only 22.2-49% and specificity of 64.7 – 97.4%.(117, 118) Dynamic “4D” 320 slice CT of the larynx has also

been described as a technique for the diagnosis of VCD with a median reduction in calculated laryngeal luminal area during expiration of 78.2% (range 48.2-92.5%) compared to 10.4% (range 4.7-30%) among patients without VCD, although the sensitivity of this test and correlation with laryngoscopy may be near 50% (119, 120).

Laryngoscopy is important to perform to identify any structural or neurological abnormalities, as well as looking to capture paradoxical adduction of the laryngeal inlet. However, due to the episodic nature of symptoms, it may often be normal at rest in the absence of exacerbating factors or triggers.(121) Thus combining laryngoscopy with provoking agents or challenge testing may increase diagnostic yield. Exercise is a common trigger for VCD and asthma. Laryngoscopy may be performed on a stationary bicycle, with a treadmill or while climbing up stairs.(122, 123) In one study, 92% of patients with an initially normal laryngoscopy had abnormalities detected following exercise.(103) Another study demonstrated VCD with exercise challenge in 66.6% of those with initially normal laryngoscope findings.(124) In a study using odour challenge prior to laryngoscopy, identification of paradoxical vocal fold movement increased from 47% with quiet respiration to 67% following odour provocation.(125) Other bronchial provocation agents have been used to trigger VCD and may also be useful in excluding uncontrolled asthma or bronchial hyperreactivity as a cause for the patient's symptoms. These include methacholine (126, 127), histamine(115), 4.5% hypertonic saline(106) and mannitol.(128)

VCD does not respond to standard asthma treatment, and may in fact be exacerbated by inhaled corticosteroids. Speech pathology is the mainstay of treatment and is multifaceted, and may comprise education, reduction of laryngeal irritation strategies, rescue breathing exercises, counselling, relaxation techniques and inspiratory muscle training.(107) Other treatment options include continuous positive airway pressure (CPAP) to reduce expiratory flow, reduce the effort required for inspiration and open the glottis (129) and botulinum

toxin injections,(130) however the data for these interventions is limited to small studies. Neuromodulating medications have also been used in chronic cough, but more studies are required to determine effectiveness in VCD.

Knowledge gaps

There are no agreed diagnostic guidelines for the identification of vocal cord dysfunction within a difficult-to-treat asthma population. Clinical risk factors that may predict the presence of vocal cord dysfunction are also not well described. Further research into how clinicians should evaluate and address VCD within this population is needed, and these questions are addressed in *Chapters Five and Six* of this thesis.

Chronic cough hypersensitivity and difficult-to-treat asthma

Cough in patients with difficult asthma is a common symptom that significantly impacts quality of life and disease severity. In a study of patient weighting of importance of asthma symptoms, chronic cough was ranked highly by nearly 60% of patients as the most troublesome affecting quality of life, even more so than wheeze, dyspnoea or sleep disturbance.(131) In a Danish study of patients from a general population with asthma and chronic cough, they had worse respiratory symptoms, increased health care use, lower lung function and higher levels of inflammatory biomarkers. More specifically, in patients with asthma and chronic cough, there was increased wheezing (70% vs 54%, $p<0.05$), dyspnoea (74% vs 49%, $p>0.005$), sputum production (59% vs 14% $p<0.005$) and GP visits (60% at least three visits vs 45%, $p<0.05$). FEV_1 was also more likely to be less than 60%.(132)

It has previously been assumed that cough in asthma was due to bronchial hyperresponsiveness with (eosinophilic) airway inflammation, however it may more so be

associated with chronic cough hypersensitivity or dysfunctional airway innervation. The cough reflex is a neuronally mediated pathway and many patients with asthma may have features of hyperresponsiveness and hypersensitivity of this reflex, which is often refractory to standard asthma treatments, including ICS.(133)

In a study using capsaicin to evoke cough in asthma patients, patients with asthma, particularly non-atopic, type two 'low' asthma, had exaggerated cough responses consistent with neuronal dysfunction.(89) Increased capsaicin sensitivity in patients with severe asthma was also associated with worse clinical outcomes, including frequent exacerbations and poor asthma control.(134)

Chronic cough hypersensitivity is characterised by increased sensitivity to irritants such as smoke, exhaust fumes, chemical smells and odours (hypertussia) or cough that is triggered by usually nontussive stimuli such as exercise and cold air (allotussia).(135) The underlying pathophysiology is thus a sensory neuronal laryngeal dysfunction which also similarly underlies vocal cord dysfunction. Indeed VCD has been reported in 56% of patients with chronic cough, which was also found to be associated with reduction in inspiratory airflow, significant extrathoracic airway hyper-responsiveness and reduced quality of life.(136)

Given the inadequacy of inhaled corticosteroids in improving this symptom, alternative treatment pathways are required. Neuromodulator therapies such as gabapentin, amitriptyline and pregabalin are effective among some patients with chronic cough and are currently undergoing investigation for efficacy in VCD populations.(137, 138) Speech language therapy interventions have also been shown to be useful for patients with both chronic cough and VCD.(139)

Knowledge gaps

Objective clinical investigations to guide accurate diagnosis of chronic cough hypersensitivity and to differentiate it from cough associated with airway inflammation and bronchial hyperresponsiveness are essential, particularly when appropriate treatments are so distinct. Dry powder mannitol is used for indirect bronchoprovocation testing with high diagnostic specificity for asthma.(30) Mannitol challenge induces an increased cough response among patients with asthma and chronic cough,(140) and can also provoke VCD(128). However, the laryngeal and bronchial components of cough and characterisation of patients with abnormal cough responses to mannitol have not been defined. This knowledge gap is addressed in *Chapter Seven* of this thesis.

Chapter Two: Methods

In this thesis, the methods used for each of the studies included are reported in detail within each paper. To summarise, this thesis used data from three study populations, which will be described in this chapter. To improve the clinical applicability and external validity of the results of the studies included, participants were recruited from clinics rather than a clinical trial population. This 'real world' approach is considered the most appropriate to answer the thesis hypotheses given that factors relating to difficult asthma, including comorbidities and non-adherence, are often exclusion criteria for controlled clinical trials. However, the limitations to this approach include reduced internal validity and risks of recall and selection bias.

Furthermore, as the study populations were obtained from a single specialist tertiary centre, findings may be less applicable to the 'real world' of patients managed in general practice or secondary care. An advantage of undertaking a single centre study means that my research questions could be efficiently investigated in the study period available for this thesis. Additionally, as the research questions posed were novel, it is still often useful to test these hypotheses in a smaller number of subjects in the first instance, before larger confirmatory studies are conducted. However, a considerable limitation of the single centre study is the smaller sample size, which decreases statistical power and increases the risk of type II error. Further discussion on the strengths and limitations of the methodology of this thesis is presented in Chapter eight.

1. *Patients assessed through the Alfred Difficult Asthma Protocol*

The Alfred hospital is a tertiary referral center in Melbourne, Victoria, Australia. Prospective data captured from patients referred to the difficult asthma clinic at this hospital was used in study one (Chapter three) addressing aim one (prevalence of non-adherence in a difficult asthma population), study two (Chapter four) addressing aim two (use of entropy metrics to

evaluate asthma inhaler adherence and their association with asthma outcomes) and study four (Chapter six) addressing aim four (identification of clinical factors associated with the comorbidity of laryngeal dysfunction and paradoxical vocal fold movement. This clinic began as a protocolised systematic assessment in 2014 following an audit of our general asthma clinic which suggested that 13% of patients with asthma would likely benefit, even after specialist respiratory and allergist care. Reasons for referral to the difficult asthma protocol included: diagnostic dilemmas, poor symptom control, frequent or severe asthma exacerbations, poor lung function or patient factors. In previous cross-sectional analyses, the patients presenting to the clinic were found to have a high burden of symptoms, poor asthma-related quality of life and high steroid requirements. Many were also found to have extra-pulmonary comorbidities, such as vocal cord dysfunction, that had not previously been diagnosed.(16)

The systematic assessment took place over three visits in a six-month period and data were captured by patient-completed standardised questionnaires prior to each visit and during the visit by the assessing clinician (Figure 2). Questionnaires for screening comorbidities were chosen based on ease of use, presence of a cut-off score and adequate validation. Radhakrishna and colleagues identified that the addition of these questionnaires to the protocol significantly improved detection of comorbidities.(92) All questionnaires were used with permission of the authors (table two). Electronic monitoring devices (Smart Inhaler™, Adherium) were also applied to patient preventer inhalers with these data downloaded at visit two. These data were used in study one (chapter three) and study four (chapter five). Data were managed within the secure web application Research Electronic Data Capture (REDCap) via an electronic platform known as the Severe Asthma Global Evaluation (SAGE) (Figure 3).(141, 142)

In total, SAGE comprises 6 modules. A questionnaire module, completed by patients electronically prior to assessment, an asthma module comprising history of asthma and its severity, asthma triggers, phenotyping and asthma medications, a comorbidity module to evaluate 8 commonly associated comorbidities, an asthma management module summarising a comprehensive asthma and comorbidity patient management plan, a nurse-educator module – including assessment of adherence and electronic monitoring, formal asthma education and asthma action plan delivery, and a panel discussion module for multidisciplinary team input.(142)

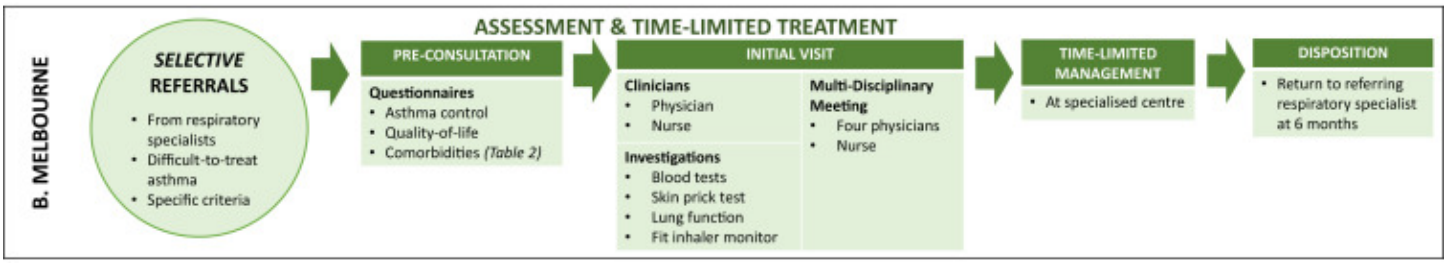

Figure 3. Systematic assessment protocol for patients referred to the Alfred hospital Difficult Asthma Protocol. Figure adapted from Hew et al JACIP 2020, used with permission, license number 5184191168848 *(143). Questionnaires used outlined in table 2.*

Table 2. Asthma and Comorbidity Questionnaires.

	Questionnaire		Goal
Asthma	ACQ6	< 1.5 controlled, MCID 0.5	Low Score
	ACT	< 15 poor control, MCID 3	High Score
	GINA	> 3 poor control	Low Score
	AQLQ	Range: 1 - 7, MCID 0.5	High Score
	SAQ	Range: 1 - 7, MCID 0.46 *	High Score
Sinonasal Disease	SNQ	> 1 sinonasal disease	Low Score

Allergic Rhinitis	RCAT	≤ 21 poor control	High Score
Chronic Rhinosinusitis	SNOT-22	> 40 poor control, MCID 8.9	Low Score
GORD	GERD Q	0-2 = 0%; 3-7 = 50%; 8-10 = 79%; 11-18 = 89% likelihood	Low Score
Sleep Apnoea	BERLIN	0-1 Low Risk, 2-3 High Risk	Low Score
Anxiety	HADS-A	< 7 Normal, 7-11 Borderline, >11 Abnormal	Low Score
Depression	HADS-D	< 7 Normal, 7-11 Borderline, >11 Abnormal	Low Score
Breathing Pattern Disorder	Nijmegen	>23 Hyperventilation Syndrome	Low Score
Vocal Cord Dysfunction	VCD-Q	34-40 Intermediate; >40 High Probability	Low Score
	Pittsburgh Index	>4 VCD	Low Score

Table 2. Questionnaires used in the difficult asthma clinic systematic assessment protocol. Abbreviations: ACQ6 – Asthma control questionnaire 6, ACT- Asthma control test, GINA – Global initiative for asthma, AQLQ – asthma quality of life questionnaire, SAQ- severe asthma questionnaire, SNQ- Sinonasal questionnaire, RCAT – rhinitis control assessment test, SNOT- sinonasal outcome test, GERD-Q – Gastroesophageal reflux disease questionnaire, HADS A and D - Hospital anxiety and depression scale, VCD-Q – Vocal cord dysfunction questionnaire.

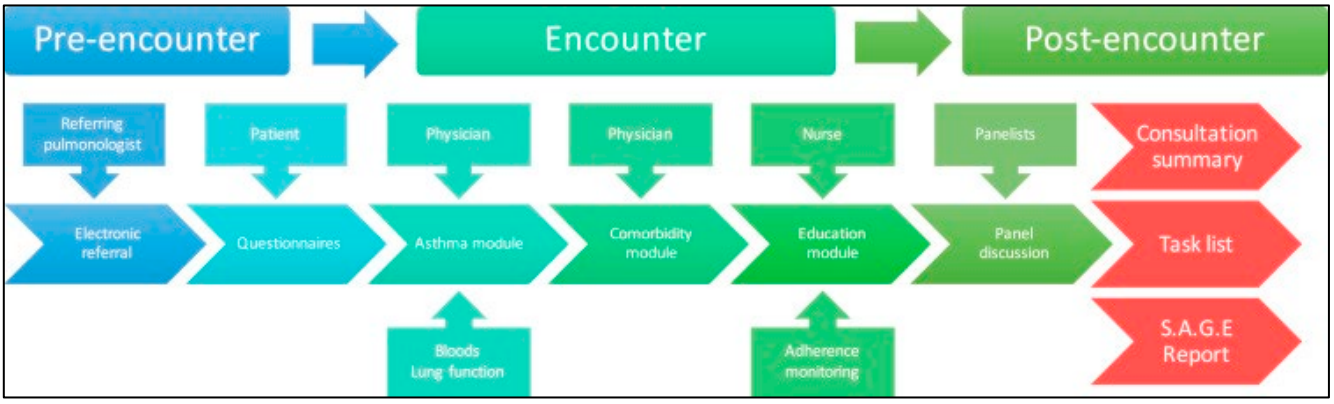

Figure 4. Protocolised assessment for difficult to treat and severe asthma incorporating an electronic system, with outputs in red. SAGE – Severe Asthma Global Evaluation. Copyright licence not required. (142)

Cross-sectional and longitudinal analyses of this patient population have been previously reported, prior to or during this thesis.(4, 16, 92, 95, 144) In an analysis of 90 patients referred for the protocol, comorbidities including obesity as measured by body mass index, dysfunctional breathing and chronic rhinosinusitis, were associated with worse asthma outcomes including high exacerbation rates, poorer asthma control and poorer asthma quality of life. Patients with vocal cord dysfunction also had a poorer quality of life. (95) Using targeted comorbidity interventions identified through the protocol improved control of comorbidities such as chronic rhinosinusitis and dysfunctional breathing, as well as improving asthma control, quality of life and reducing frequency of exacerbations.(4) Evaluation of the systematic assessment protocol has also been shown to halve patient requirements for oral corticosteroids, and was also associated with a 64% reduction in asthma exacerbations.(144)

2. *Patients assessed through the Alfred Middle Airway Clinic*

Study three (Chapter five), addressing aim three, used data prospectively collected from patients attending a dedicated ''middle airway'' clinic, which was established in 2015 at Alfred health specifically for the diagnosis and management of patients with both suspected vocal cord dysfunction (or inducible laryngeal obstruction) and asthma. Patients had been referred by directly by the difficult asthma clinic or by respiratory, allergy or ear, nose and throat specialists. These patients underwent a systematic, multidisciplinary assessment over two visits which also included a series of questionnaires (table 3) completed by patients prior to each visit. An online database was again created using REDCap with a standardised, electronic clinical template.(141)

Questionnaire	**Cut-Off Score**	**Goal**
VCD-Q	34-40 Intermediate, >40 High Probability	Low Score
Newcastle Laryngeal Hypersensitivity Score	>17.1 Normal, MCID 1.75	High Score
Dyspnoea Index	>10 Abnormal	Low Score
Asthma Control Test	< 15 Poor Control, MCID 3	High Score
CSS-SHR	>43 Hypersensitivity	Low Score
GERD-Q	0-2 0%; 3-7 = 50%; 8-10 = 79%; 11-18 = 89% likelihood	Low Score
HADS A	< 7 Normal, 7-11 Borderline, >11 Abnormal	Low Score
HADS-D	< 7 Normal, 7-11 Borderline, >11 Abnormal	Low Score
Nijmegen Questionnaire	>23 Hyperventilation Syndrome	Low Score

Rhinitis control (RCAT)	< 12 Poor Control	High Score
Sinonasal disease (SNQ)	>1 Sinonasal Disease	Low Score
SNOT-22	>40 Poor Control, MCID 8.9	Low Score

Table 3. Questionnaires used during Middle Airway Clinic. VCD-Q – Vocal cord dysfunction questionnaire test, CSS-HSR – chemical sensitivity scale for sensory hyperreactivity, GERD-Q – Gastroesophageal reflux disease questionnaire, HADS A and D, Hospital anxiety and depression scale, RCAT – rhinitis control assessment test, SNQ- Sinonasal questionnaire, SNOT-sinonasal outcome test.

Clinical assessments were multidisciplinary and were undertaken by respiratory physicians with experience in the management of severe asthma and middle airway disorders, as well as otolaryngologists (ENT) (from 2015 to mid-2017) and speech pathologists. From 2015 to June 2017, patients were seen at two consecutive appointments with the respiratory physician initially, followed by an ENT assessment with attendant speech pathologist. All patients underwent lung function testing.

Objective confirmation of vocal cord dysfunction was sought by laryngoscopic observation of paradoxical vocal fold motion (PVFM). Laryngoscopy was performed by expert respiratory physicians or otolaryngologists (prior to June 2017). If normal vocal fold movements were observed at baseline, provocation strategies were used including dry powder mannitol or odour challenge. High interrater agreement of post-mannitol laryngoscopy findings between respiratory specialists and laryngologists has been previously reported ($\kappa = 0.696$, 95% confidence interval: 0.324-1, p=0.006).(128) After June 2017, due to these results and changes in available personnel, patients were evaluated and investigated by respiratory physicians followed by speech pathologists. Targeted referral to ENT was made when laryngeal pathology (other than PVFM) is identified on laryngoscopy. Speech pathology assessment included voice evaluation, measurement of phonation time, vocal hygiene, and swallow assessment.

Objective confirmation of asthma was sought by demonstration of variable airflow obstruction either by bronchodilator response (increase by greater than or equal to 200mL and 12%), peak flow variability of more than 15% or positive bronchial provocation challenge test (greater than or equal to a drop in 15% of FEV_1 with cumulative mannitol dose less than 635mg). If asthma was excluded, asthma medications such as inhaled corticosteroids, were ceased as appropriate. If vocal cord dysfunction was identified, patients were referred for speech pathology interventions including adjustment of maladaptive posture, respiratory retraining, educational counselling, and behavioural breathing exercises.

3. *Patients undergoing mannitol bronchoprovocation through the Alfred Lung Function Laboratory*

Study five (chapter seven), addressing aim five, used data collected from patients attending the lung function laboratory at Alfred health for bronchial provocation challenge testing with mannitol between November 2015 and July 2017. The lung function laboratory at Alfred health is a large laboratory accredited by the Thoracic Society of Australia and New Zealand. The laboratory performs a wide range of respiratory function tests according to internationally accepted American Thoracic Society and European Respiratory Society protocols and averages 10,000 patient encounters per year. Referrals are received mostly from within the Alfred health Respiratory department, but also from other departments within the hospital and from the community and primary care. During the study period, 245 bronchial provocation challenges were performed (90 were included). Patients recruited to the study completed the 14-item Newcastle Laryngeal Hypersensitivity Questionnaire (113) and their coughs during the procedure were recorded and manually counted. The mannitol challenge was performed following the recommendations of the manufacturer (Aridol™, Pharmaxis, NSW Australia).

A summary of the three cohorts used in this thesis is presented in Table 4.

All studies were conducted with the approval of the Alfred Health Ethics Committee and/or Monash University Human Research Ethics Committee (MUHREC) (Available in the Appendix). Patients provided written informed consent when required by ethics.

	Difficult Asthma Clinic cohort	**Middle Airway Clinic cohort**	**Lung function laboratory cohort**
Data collection	Prospective	Prospective	Prospective
Patients	Patients with difficult-to-control asthma, referred by respiratory physicians.	Patients with suspected comorbid asthma and/or vocal cord dysfunction, referred by respiratory physicians, allergists, immunologists or ear, nose and throat specialists.	Patients presenting for bronchial provocation testing with mannitol for investigation of chronic cough or suspected asthma. Patients referred by respiratory physicians, general physicians, and general practitioners in primary care.
Study visits and time period	Protocolised systematic assessment over three visits in a six-month period.	Protocolised systematic assessment over two visits in a six-month period.	One visit
Number of Questionnaires used	15	13	1
Investigations and interventions	Lung function testing including FeNO, blood testing, allergy testing, inhaler electronic monitoring (EMD).	Lung function testing (Spirometry), laryngoscopy +/- provocation, speech language therapy assessment.	Bronchial provocation with mannitol (Aridol™). Cough counting and recording.
Data Analyses	EMD data for adherence assessment. Entropy measurement. Descriptive statistics, univariate analyses and multivariable regression analyses.	Descriptive statistics and univariate analyses.	Descriptive statistics, univariate comparisons, multivariable logistic regression analysis and cluster analysis.
Chapter(s) of thesis	3, 4, 6	5	7

Table 4. A summary of the three patient cohorts studied in this thesis. EMD – Electronic monitoring device.

SECTION I: Medication Adherence in Difficult Asthma

Introduction

This section contains two chapters addressing poor medication adherence as a potentially modifiable patient factor underlying difficult-to-treat asthma. As described in Chapter one, non-adherence to inhaled corticosteroids in asthma is associated with poor asthma outcomes including increased risk of exacerbations requiring high doses of systemic corticosteroids, hospitalisation and death.(37) While it is likely that the majority of patients with asthma will respond well and be able to achieve satisfactory disease control on inhaled corticosteroids alone, a small proportion continue to have biologically severe asthma, and would greatly benefit from the more recently available targeted treatments including monoclonal biological therapies and bronchial thermoplasty.

Such treatments, while effective, come with significant costs to the health system and are not without their own risk of adverse effects. Therefore, in order to accurately identify those patients that would genuinely benefit from these therapies, the development of strategies to optimise medication adherence to preventer inhalers in this population are a priority. To develop these strategies, it must first be understood how prevalent suboptimal adherence to inhaled medications in difficult asthma is, as well as how well health care professionals can detect it. This knowledge gap is addressed in Chapter 3, in the study titled "*Nonadherence in the era of severe asthma biologics and thermoplasty."*

Furthermore, when poor inhaler adherence is suspected, specific measurement of inhaler behaviour to confirm non-adherence as well as quantify asthma risk would be valuable to identify patients most at risk of poor asthma outcomes. Current averaged measurements of inhaler use do not adequately describe patient inhaler behaviour, and more reliable and

objective measurements are required. This knowledge gap is addressed in Chapter 4 in the study titled "*Dynamics of inhaled corticosteroid use are associated with asthma attacks."*

Chapter Three: Prevalence of Medication Non-Adherence within Difficult Asthma

3.1 Introduction

This study examined the adherence of patients presenting to the difficult asthma clinic at Alfred health via the use of electronic monitoring devices (EMD). The prevalence of non-adherence and suspected non-adherence is reported, and related to suitability for novel therapies including biologics and bronchial thermoplasty. In addition, the sensitivity and specificity of health care professional assessment of patient adherence is assessed and compared to the "true" rates of adherence captured by EMD.

Lee J, Tay TR, Radhakrishna N, Hore-Lacy F, Mackay A, Hoy R, Dabscheck E, O'Hehir R, Hew M. Non-adherence in the era of severe asthma biologics and thermoplasty. *European Respiratory Journal*; Jan 2018, 1701836; **DOI:** 10.1183/13993003.01836-2017.

Nonadherence in the era of severe asthma biologics and thermoplasty

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Preventer adherence is underrecognised and must be confirmed objectively prior to initiating novel asthma treatment http://ow.ly/Kc1X30iysYD

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ABSTRACT Nonadherence to inhaled preventers impairs asthma control. Electronic monitoring devices (EMDs) can objectively measure adherence. Their use has not been reported in difficult asthma patients potentially suitable for novel therapies, *i.e.* biologics and bronchial thermoplasty.

Consecutive patients with difficult asthma were assessed for eligibility for novel therapies. Medication adherence, defined as taking >75% of prescribed doses, was assessed by EMD and compared with standardised clinician assessment over an 8-week period.

Among 69 difficult asthma patients, adherence could not be analysed in 13, due to device incompatibility or malfunction. Nonadherence was confirmed in 20 out of 45 (44.4%) patients. Clinical assessment of nonadherence was insensitive (physician 15%, nurse 28%). Serum eosinophils were higher in nonadherent patients. Including 11 patients with possible nonadherence (device refused or not returned) increased the nonadherence rate to 31 out of 56 (55%) patients. Severe asthma criteria were fulfilled by 59 out of 69 patients. 47 were eligible for novel therapies, with confirmed nonadherence in 16 out of 32 (50%) patients with EMD data; including seven patients with possible nonadherence increased the nonadherence rate to 23 out of 39 (59%).

At least half the patients eligible for novel therapies were nonadherent to preventers. Nonadherence was often undetectable by clinical assessments. Preventer adherence must be confirmed objectively before employing novel severe asthma therapies.

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Introduction

"Difficult asthma" is a term referring to patients who remain uncontrolled despite treatment at Steps 4 and 5 of the Global Initiative for Asthma (GINA) guidelines [1]. The overall prevalence of difficult asthma has been estimated at between 10% and 17% of asthma patients [1, 2].

A major contributor to difficult asthma is unsuppressed airway inflammation consequent to relative corticosteroid insensitivity [3]. In such patients, novel treatment options are now licensed for specific disease phenotypes, specifically monoclonal biologics targeting IgE (omalizumab) and the interleukin (IL)-5 pathways (mepolizumab, reslizumab) or bronchial thermoplasty [4–7]. Such treatments are expensive and should only be considered after standard therapy (including high-dose inhaled corticosteroids (ICSs), usually in combination with a long-acting bronchodilator) has been optimised [8–11].

Medication adherence can be defined as "the degree to which the medication use of the patient corresponds to the prescribed regimen" [12, 13]. Patient nonadherence to medications can thus vary over time, and can be both intentional (*e.g.* due to fear of side-effects) and nonintentional (*e.g.* due to cost or forgetfulness). Identifying and addressing nonadherence to inhaled respiratory medications has been identified as an urgent priority for international policy makers [14]. Medication nonadherence is particularly prevalent in difficult asthma, with previous estimates of nonadherence of ∼50% by prescription refills [15–17]. Assessing prescription refills can be challenging if there are multiple prescribers and dispensing pharmacies. In clinical practice, preventer adherence is usually assessed subjectively by the treating health professional or based on patient self-report, which are both notoriously inaccurate [18]. However, inaccurate subjective assessments can have significant consequences: a large proportion of patients with difficult asthma also have severe biological asthma and poorer clinical outcomes are seen in those who are nonadherent to inhaled preventers [19]. Additionally, if nonadherence remains undetected in difficult asthma, patients may proceed inappropriately to targeted biological therapy or thermoplasty [9].

Detailed objective measurements of adherence may now be obtained by electronic monitoring devices (EMDs) [20–22]. These devices can be placed on the patient's preventer inhaler on initial contact and data downloaded at the next clinic visit. EMDs have been used to examine nonadherence in asthma, but data regarding their utility in difficult asthma are limited.

We hypothesised that nonadherence in difficult asthma remains a significant issue in the era of novel severe asthma therapies. We used an EMD to objectively measure preventer nonadherence in difficult asthma and compared this with structured, albeit subjective, clinician assessment. We specifically examined the rate of nonadherence among patients suitable for anti-IgE and anti-IL-5 biologics or thermoplasty.

Materials and methods

Patients were referred with difficult asthma if their treating respiratory or allergy specialists had difficulty with their asthma management. Reasons for referral (usually multiple) were diagnostic dilemma, poor symptom control, frequent or severe exacerbations, poor lung function, or patient factors, including comorbidities and suspected nonadherence [23].

Patients undergo systematic evaluation over 6 months in three visits to: confirm the diagnosis of severe asthma, to identify and address exacerbating triggers or comorbidities (including anxiety and depression, vocal cord dysfunction, dysfunctional breathing, gastro-oesophageal reflux disease, obstructive sleep apnoea, allergic rhinitis, and chronic rhinosinusitis), and to determine the inflammatory phenotype to facilitate selection of optimal medical therapy, including targeted biologics [24]. As previously described, the assessment process is supported by questionnaires for the assessment of the patient's asthma control (Asthma Control Test (ACT)) [25] and quality of life (Asthma Quality of Life Questionnaire (AQLQ)) [26], as well as a comorbidity questionnaire battery, an electronic platform and a panel discussion for each patient [27–29]. Permission was obtained for all administered questionnaires.

Our centre prescribes asthma biologics [30], but does not perform bronchial thermoplasty.

This study included consecutive patients who entered the difficult asthma protocol between May 1, 2015 and December 31, 2016. The study was approved by the Alfred Health Ethics Committee (285/15).

Eligibility for novel asthma therapies

For this study, patients were categorised as eligible for biologics if they met American Thoracic Society (ATS)/European Respiratory Society (ERS) criteria for severe and uncontrolled asthma [24], and had either an eosinophilic (blood eosinophil count $\geqslant 0.3\times10^9$ L^{-1}; suitable for anti-IL-5 therapy) or allergic phenotype (serum IgE level $\geqslant 30$ $kU{\cdot}mL^{-1}$ and sensitisation to an aeroallergen based on skin testing or serum-specific IgE; suitable for anti-IgE therapy).

Patients were categorised as eligible for thermoplasty based on entry criteria for the Research in Severe Asthma (RISA) trial [7]; high-dose ICS/LABA inhalers, pre-bronchodilator forced expiratory volume in 1 s >50% predicted, positive bronchoprovocation or bronchodilator response, uncontrolled asthma and no current smoking nor prior smoking history of ⩾10 pack-years.

Adherence assessment

At the first clinic visit, the difficult asthma protocol physician would explain to each patient that the routine procedure for all patients was to provide an EMD for objective monitoring of asthma management if their inhaler device was compatible. Language was aimed to be neutral and nonjudgemental. A Smartinhaler device (Adherium, Auckland, New Zealand) was attached to the patient's preventer inhaler. EMDs were available for a variety of ICS or ICS/long-acting β-agonist (LABA) combination inhalers, specifically metered dose inhalers and dry powder devices for Flixotide (fluticasone propionate), Seretide (salmeterol and fluticasone propionate), Pulmicort (budesonide) and Symbicort (budesonide and formoterol). EMDs were not available for other ICS or ICS/LABA combination inhalers. The EMD was able to record the timing of the doses taken according to the number prescribed for morning or evening.

Data were downloaded at the scheduled follow-up visit at the 2-month time-point. Data collected included the date, time and number of actuations, preventing "dose dumping".

For the purposes of this study, the patient was considered adherent if >75% of prescribed doses were actuated at the times they were prescribed, based on an increased risk of exacerbations reported below this cut-point [31]. Day-to-day adherence rate was not reported. Patients were defined as possibly nonadherent if they declined to have the device added to their inhaler or did not return the device despite reminders.

Adherence was also assessed at the first visit by the referring specialist, the patient, the difficult asthma clinic respiratory physician and a clinical asthma nurse. Referring specialists also completed a standardised referral form and could indicate whether they felt the patient was nonadherent.

At the first clinic visit during systematic evaluation, self-reported adherence was documented if the patient agreed with the following statement "I follow my medication plan".

The difficult asthma clinic specialist assessed patient adherence using the components of the validated Adult Asthma Adherence Questionnaire [32], including specific questions surrounding forgetfulness, a perception that preventer treatment was unnecessary, fear of side-effects and cost. Following the assessment, physicians were prompted to estimate inhaler adherence as <50%, 50–75% or >75%.

At the first clinic visit, all patients underwent clinical asthma nurse assessment and education to address the patient's understanding of asthma, inhaler technique and adherence. The asthma nurse fitted the electronic device to the patient's inhaler. Following assessment, nursing staff were prompted to estimate adherence as "good", "partial" or "poor". Patients estimated to have "partial" or "poor" adherence were considered nonadherent.

Statistical analysis

Data analysis was performed using SPSS version 22 (IBM, Armonk, NY, USA). Categorical variables are presented as percentages (frequency) and continuous variables are presented as mean or median values with standard deviation and/or range. T-test for comparison of means and Fisher's exact or Chi-squared tests for comparison of proportions were performed where appropriate.

Results

During the study period, 71 consecutive patients underwent systematic evaluation. Two patients did not have asthma, had their inhalers discontinued and were excluded from further analysis. Baseline characteristics of the remaining 69 patients are presented in table 1.

In this difficult asthma cohort, poor asthma control and quality of life were reflected in the average ACT and AQLQ scores. 86% of patients were on GINA Step 4 or 5 asthma treatment. 59 out of 69 (85%) patients had severe asthma, all of whom had uncontrolled asthma (as defined by the ERS/ATS guidelines) [24].

Eligibility for biologics and thermoplasty

30 (43.5%) patients were eligible for anti-IgE therapy and 22 (31.9%) for anti-IL-5 therapy, with 38 (55%) potential candidates for either. 26 patients (37.7%) were eligible for thermoplasty. In total, 47 (68%) patients were eligible for a biologic or thermoplasty, or both (figure 1).

EMD adherence assessment

69 patients were considered for an EMD. The flow of patients through each stage of the study is shown in figure 2.

Adherence outcomes for all 69 difficult asthma patients are shown in figure 3a. "Unknown adherence": adherence status could not be objectively assessed in 13 patients; 10 did not use a preventer with a compatible EMD and three returned a device which malfunctioned. "Possible nonadherence": another 11 exhibited behaviour suggestive of nonadherence; two patients refused the EMD and nine did not return the device, some of whom reported the device as lost. "Confirmed nonadherence": of 45 patients who returned a device with usable data, 20 (44.4%) were nonadherent; mean±SD rate of nonadherence was 51.4±20.8% (interquartile range (IQR) 38–65%). "Confirmed adherence": 25 out of 45 (55.6%) patients had documented preventer adherence of >75%; mean±SD rate of adherence was 89±6.3% (IQR 84–95%).

If patients with confirmed and possible (those who refused or did not return the EMD) nonadherence were grouped together, the overall nonadherence rate increased to 31 out of 56 (55.3%) patients.

Confirmed nonadherent patients had higher mean serum eosinophils (0.42×10^9 *versus* 0.22×10^9 L^{-1}; $p<0.05$) than confirmed adherent patients. There were no other significant associations of other clinical features that differed between adherent and nonadherent patients (table 2). The mean ICS dose (μg fluticasone equivalent) was not significantly different in patients who were adherent (982 μg) or nonadherent (850 μg).

TABLE 1 Baseline characteristics

Subjects	69
Age years	52±14.2 (19–76)
Female	41 (59.4)
Smoking status	
Never-smoker	46 (66.7)
Ex-smoker	22 (31.9)
Current smoker	1 (1.4)
BMI kg·m^{-2}	30±6.9
Early-onset asthma <18 years	34 (49.3)
Pre-bronchodilator FEV_1 % pred	62±20.2
Change in FEV_1 % pred following bronchodilator	14.2±15
FEV_1/FVC %	61±15.4
Airflow obstruction at baseline (FEV_1 <80%, FEV_1/FVC <70%)	41 (59.4)
Variable airflow obstruction demonstrable	61 (88.4)
⩾12% and ⩾200 mL improvement in FEV_1 following bronchodilator	47 (77)
>12% variability in peak flow charting over 2 weeks	12 (19.7)
Positive bronchial provocation challenge test with mannitol	2 (3.3)
Blood eosinophils ×10^9 L^{-1}	0.33±0.33 (0–1.73)
Eosinophils ⩾0.3×10^9 L^{-1}	28 (40.6)
***F*ENO ppb**	36±31.2 (4–137)
IgE kU·L^{-1}	524±1006 (2–4880)
Atopic[#]	47 (68.1)
ACT score[¶]	13.6±5.19
AQLQ score[+]	4.19±1.4
Exacerbations in last 12 months requiring oral (>3 days or increase in 20 mg from baseline prednisolone dose) or intravenous corticosteroids	5±4.7 (0–30)
Frequency of asthma exacerbations in 12 months	
0	4 (5.8)
1	8 (11.6)
2	2 (15.9)
⩾3	46 (66.7)
On ICS/LABA combination	66 (95.7)
Total ICS dose μg fluticasone equivalent	992±538 (0–3200)
On OCSs	17 (24.6)
Total OCS dose mg	8.7±6.23 (1–25)
Severe asthma by ATS/ERS guidelines	59 (85.5)
Anxiety and depression[§]	24 (35)

Data are presented as n, mean±SD (range), mean±SD or n (%). BMI: body mass index; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; *F*ENO: exhaled nitric oxide fraction; ACT: Asthma Control Test; AQLQ: Asthma Quality of Life Questionnaire; ICS: inhaled corticosteroid; LABA: long-acting β-agonist; OCS: oral corticosteroid; ATS: American Thoracic Society; ERS: European Respiratory Society. [#]: positive skin prick test or serum-specific IgE to commonly tested aeroallergens; [¶]: <15 indicating poor control; [+]: out of 7, high score indicating better quality of life; [§]: diagnosis based on presence of clinical symptoms and Hospital Anxiety and Depression Scale [33] score ⩾11 or known history on treatment.

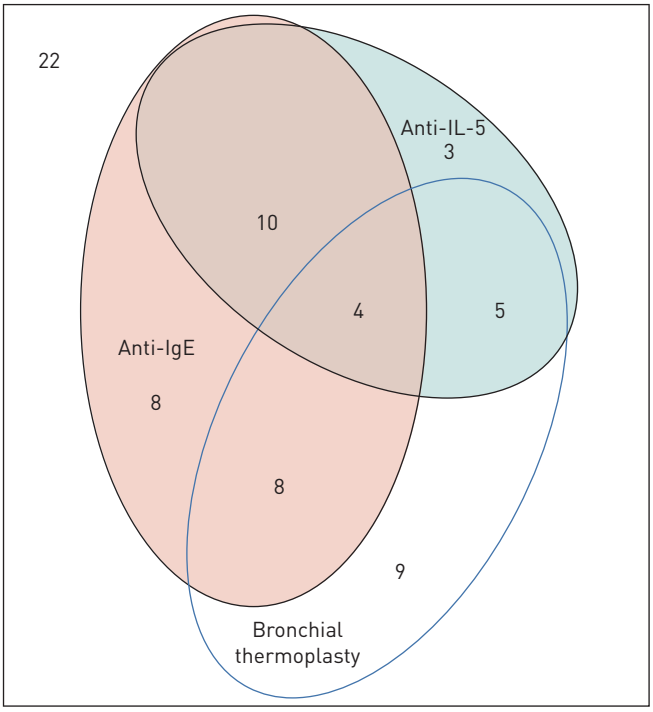

FIGURE 1 Patients eligible for novel asthma therapies: biologics and bronchial thermoplasty (47 out of 69). IL: interleukin.

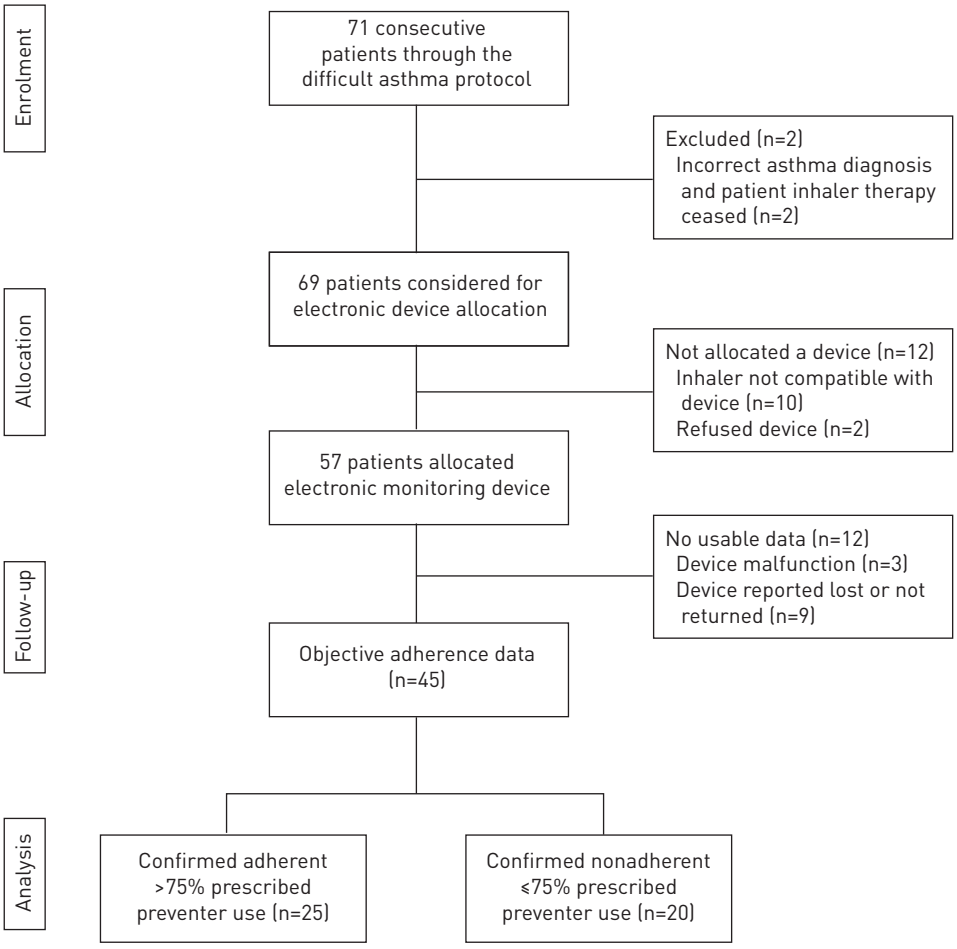

FIGURE 2 Patient flow through each stage of the study.

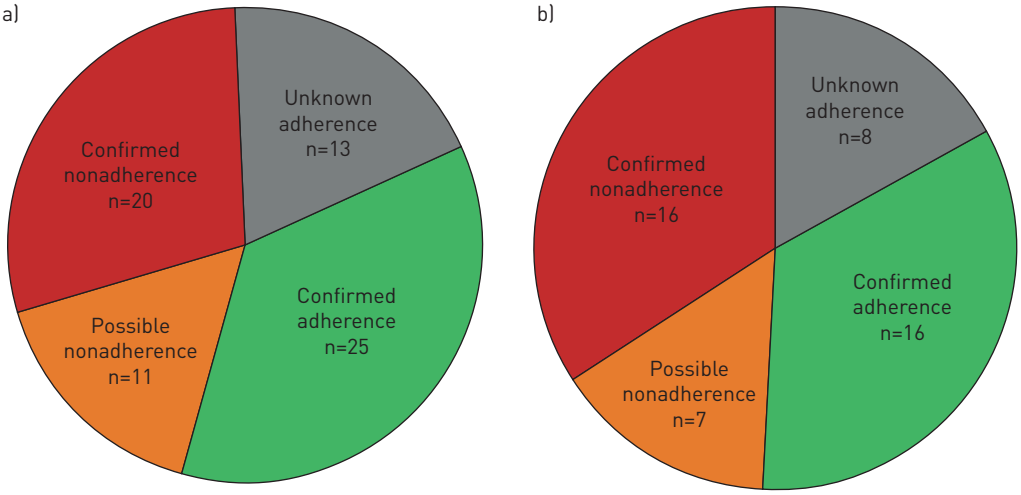

FIGURE 3 Patients with unknown adherence, confirmed adherence, possible nonadherence and confirmed nonadherence in a) all 69 difficult asthma patients and b) the 47 patients eligible for novel asthma therapies (biologics or thermoplasty).

Among 47 patients either eligible for biologics or bronchial thermoplasty, adherence could not be assessed in eight (EMD-incompatible inhaler or device malfunction) and nonadherence was possible in seven (refused or did not return the EMD). 32 patients returned usable EMD data, of whom 16 out of 32 (50%) had confirmed nonadherence on EMD assessment. Combining confirmed and possible nonadherent populations gave a nonadherence rate among those eligible for novel therapies of 23 out of 39 (59%) patients (figure 3b).

TABLE 2 Characteristics of adherent compared with nonadherent patients

	Adherent	Nonadherent	p-value
Subjects	25	20	
Age years	54±12	54±16	NS
Female	13 (52)	11 (55)	NS
Smoking status			
Never-smoker	14 (56)	14 (70)	
Ex-smoker	11 (44)	5 (25)	
Current smoker	0 (0)	1 (5)	
BMI kg·m^{-2}	30±5	31±8	NS
Early-onset asthma <18 years	13 (52)	8 (40)	NS
Pre-bronchodilator FEV_1 % pred	65±22	60±18	NS
FEV_1/FVC %	61±17	58±12	NS
Airflow obstruction at baseline (FEV_1 <80%, FEV_1/FVC <70%)	14 (56)	13 (65)	NS
Blood eosinophils ×10^9 L^{-1}	0.22±0.21	0.42±0.34	<0.05
F_{ENO} ppb	27.22±18	41.4±30	NS
IgE kU·L^{-1}	369.5±736	551.5±1030	NS
ACT score[#]	12.2±4	13.5±6	NS
AQLQ score[¶]	4.34±1	3.99±1	NS
Exacerbations in last 12 months requiring oral (>3 days or increase in 20 mg from baseline prednisolone dose) or intravenous corticosteroids	3.5±18	2.8±2	NS
Total ICS dose µg fluticasone equivalent	982±444	850±379	NS
Total OCS dose mg	4±2	9.4±5	NS
Severe asthma by ATS/ERS guidelines	21 (84)	19 (95)	NS
Anxiety or depression[+]	11 (44)	7 (35)	NS

Data are presented as n, mean±SD or n (%). BMI: body mass index; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; F_{ENO}: exhaled nitric oxide fraction; ACT: Asthma Control Test; AQLQ: Asthma Quality of Life Questionnaire; ICS: inhaled corticosteroid; OCS: oral corticosteroid; ATS: American Thoracic Society; ERS: European Respiratory Society; NS: nonsignificant. [#]: <15 indicating poor control; [¶]: out of 7, high score indicating better quality of life; [+]: diagnosis based on presence of clinical symptoms and Hospital Anxiety and Depression Scale [33] score ⩾11 or known history on treatment.

Subjective adherence assessment

Two of the 45 patients with EMD data had nonadherence identified by the referring specialist as a reason for the patient's poor asthma control. Two patients admitted that they did not follow their prescribed medication plan. Protocol physicians identified five patients as being nonadherent (of these, two were proven to be adherent objectively), whereas asthma nurses identified seven patients as being nonadherent (of these, two were also proven to be adherent objectively) (figure 4).

Compared with the EMD, the sensitivity and specificity of physician detection of nonadherence was 15% (95% CI 3.2–37.9%) and 92% (95% CI 74–99%), respectively. The sensitivity and specificity of asthma nurse assessment was 27.8% (95% CI 9.7–53.5%) and 91.67% (95% CI 73–99%), respectively (figure 4).

Discussion

Among patients otherwise suitable for novel severe asthma therapies, our study shows an alarmingly high rate of nonadherence. Furthermore, most cases of nonadherence remained undetected despite a series of subjective clinical assessments by the referring respiratory or allergy specialist, the difficult asthma protocol specialist and asthma nursing staff. This finding emphasises the indispensable value of assessing nonadherence objectively prior to initiating biologics or performing thermoplasty for severe asthma [9].

Previous studies have shown high rates of nonadherence among difficult asthma patients by monitoring prescription refills, which gives an indication of long-term medication use [16, 34, 35]. This can be difficult to perform in health systems such as our Australian setting, where patients may obtain preventers through multiple prescribers and at multiple dispensing pharmacies of their choice. Prescription refills and other indirect methods of measuring medication adherence such as canister weights also cannot confirm that a patient actually takes their medication at the correct time. We therefore chose to use EMDs, which provide detailed information on inhaler use.

The true prevalence of nonadherence in our difficult asthma population likely lies between 44% and 55%, consistent with previous studies [15, 16]. However, the finding of even greater nonadherence among patients suitable for biologics or thermoplasty supports the premise that nonadherence is intrinsically linked to more severe markers of disease. Indeed, we found that nonadherent patients had higher peripheral eosinophil counts. A previous study also found greater sputum eosinophilia among nonadherent patients [16]. Thus, an indication for instituting severe asthma biologics may also indicate a higher risk of nonadherence. Interestingly, severity of asthma symptoms, frequency of exacerbations or poorer lung function did not seem to influence rates of medication adherence. Similarly, prevalence of anxiety and depression was not increased among patients who were found to be nonadherent.

The optimal method to assess preventer adherence remains unclear and EMDs are not infallible. An initial pilot study of the device we used recorded a mean accuracy of 97% [20]. In a clinical trial of 303 patients incorporating extensive pre- and post-study checks, there was a 6.5% malfunction rate. In addition, 3.5% of devices were lost or thrown away by participants [36]. Other trials have reported higher malfunction rates of between 15% and 20% [37, 38].

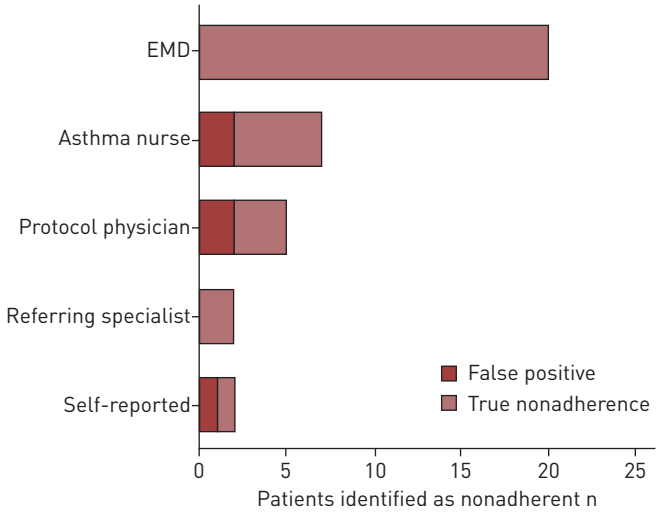

FIGURE 4 Detection of nonadherence by subjective methods in 45 difficult asthma patients with objective data from the electronic monitoring device (EMD).

In our study, adherence could not be assessed objectively in 19% of patients either due to EMD-incompatible inhalers (14.4%) or device malfunction (5.3%). Patients possibly subverted the process in another 11 cases (16%), either by refusing or not returning the device despite repeated requests; such behaviour has been reported in previous pragmatic studies [22] and in our view could also represent possible medication nonadherence. We acknowledge that some patients may have felt uncomfortable with being monitored and this may have influenced the acceptance of the device or failure-to-return rate. We chose not to implement financial incentives to return the device.

Consequently, objective EMD data was obtained in less than two-thirds of our cohort. The failure-to-return rate is higher than in previously reported clinical trials, reflecting the challenges of real-life evaluation of consecutive clinical patients, which is always more difficult than when participants have been selected for a trial. Due to cost, some guidelines have questioned the utility of EMDs in the management of asthma, outside of the research setting [39]. The EMDs cost approximately USD150 (AUD200) in 2018 prices, but this could be considered trivial in comparison with the cost of severe asthma biologics or thermoplasty procedures. However, the true cost of monitoring does go beyond the cost of devices, such as the costs of time required to manage these in the clinic setting: education of the patient, testing to reduce malfunction rates and the efforts required to ensure their return.

Self-reporting of nonadherence was unreliable and poorly sensitive in our cohort, consistent with previous studies [40, 41]. Subjective clinical assessments were also poorly sensitive for detecting EMD-confirmed nonadherence. This was despite assessment by an expert and experienced difficult asthma service, multiple assessments by three health professionals (referring physician, treating physician and asthma nurse), and the use of standardised assessments including a validated adherence tool [32]. In light of this, objective assessments are clearly indispensable to adequately detect nonadherence. Interestingly, a minority of patients who were deemed poorly adherent by clinical assessment were subsequently proven to be adherent by electronic monitoring. Although described as "false positives", this cohort may have improved their adherence behaviour in the knowledge that they were being monitored. Nevertheless, these patients may have had their access to advanced therapies inappropriately limited by the healthcare team if reliance had been placed solely on subjective measurement of adherence.

25% of our patient cohort was on oral steroids. It could be argued that patients with uncontrolled asthma despite oral steroids may require a novel therapy such as a biologic irrespective of their adherence to inhaled steroids. However, we would argue that patients nonadherent to inhaled therapy would also be likely to be nonadherent to oral medication [15]. (Assessing adherence to oral corticosteroids was beyond the scope of this study due to nonavailability of in-house serum prednisolone levels.) In addition, it is a government funding body requirement in Australia that adherence to inhaled steroids is documented prior to prescription of biologics.

The aim of our study was to detect nonadherence, not to manage it, and this study was not designed to report on longitudinal outcomes. However, an EMD can be used to provide feedback and deliver an audio reminder to the patient. These measures have been shown to improve adherence [21] and, in a paediatric population, led to fewer exacerbations requiring oral corticosteroids or hospitalisation [22]. Such benefits have yet to be shown in a difficult asthma population and would be an interesting area for future research. The overall outcomes of our 6-month, three-visit systematic assessment protocol have been previously reported [29].

This study was conducted at a single centre with an interest in difficult asthma, so the generalisability of our findings is unknown. However, the rate of nonadherence in our cohort is consistent with those reported from other difficult asthma centres in other health systems [15, 16]. Nonadherence in nonsevere asthma is even more prevalent. In a cross-sectional community study of patients with asthma (over half of which were "well controlled"), 65% of respondents were nonadherent to a preventer <4 days a week [42]. For this study, we defined eligibility for severe asthma biologics based on generic criteria and suitability for thermoplasty based on inclusion criteria for the single randomised RISA trial of thermoplasty in severe asthma [7]. However, additional criteria may apply according to local licensing authorities and funding arrangements. We further acknowledge that nonadherent patients identified by EMD may be a heterogeneous group that we were unable to stratify further in this study. Patients taking <75% of prescribed doses could be described as partially adherent and probably represent a diverse group of patients. However, this cut-off was chosen because published data demonstrates such patients are at an increased risk of adverse asthma outcomes [31]. While EMDs can confirm that an inhaler is actuated, they cannot determine if the patient actually inhaled the medication nor whether inhaler technique was satisfactory. Nevertheless, we maintain that EMD-confirmed nonadherence is a robust finding. The provision of the EMD alerted patients that their adherence was being monitored. It is possible that the degree of nonadherence might have been even greater had patients been unaware of monitoring, *i.e.* the Hawthorne effect [43].

We conclude that preventer nonadherence in difficult asthma remains disturbingly high in the era of novel (and expensive) therapies for severe asthma. There are a multitude of factors that may underlie nonadherence and we advocate for further research to be carried out in this area. Subjective assessment of adherence is highly unreliable, so objective assessments are imperative prior to initiating severe asthma biologics or performing thermoplasty.

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3.2 Summary of Findings from Chapter 3.

In this study, non-adherence to inhaled preventers among patients with difficult-to-treat asthma was highly prevalent. Among 45 patients for whom electronic monitoring data were available, 20 (44.4%) had confirmed non-adherence. If patients who were suspected of non-adherence were included, this rate increased to 55%. Most patients also had severe asthma by international criteria. Among those patients eligible for biologics and/or bronchial thermoplasty, 50% were confirmed non-adherent to their inhaled corticosteroid preventers. Additionally, self-report and clinician assessment of non-adherence was frequently inaccurate when compared to objective measurements, emphasising the importance of objective measurement of inhaler adherence prior to the commencement of advanced asthma therapies.

Chapter Four: Development of Adherence Metrics and the Use of Entropy to Predict Asthma Outcomes.

4.1 Introduction

This study presents the concept of "entropy" as a measurement of inhaler adherence and behaviour. Patient presenting to the difficult asthma clinic at Alfred health had their inhaler use tracked and monitored by electronic monitoring device. Entropy, a measure of disorder or chaos in thermodynamics, was applied as a measurement of chaotic and irregular inhaler use, and was further divided into increasing (disordered ways patients increased their daily inhaler dose) or decreasing entropy (disordered ways patients decreased their daily inhaler dose). Other adherence metrics were also measured for comparison, including mean adherence, gap durations, and time and dose area under the curve measurements. Adherence metrics were then related in multivariable analyses to baseline asthma control and quality of life, and asthma outcomes as measured by asthma attacks of all severities.

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Dynamics of inhaled corticosteroid use are associated with asthma attacks

Joy Lee[1,2]✉, Jacqueline Huvanandana[3], Juliet M. Foster[3], Helen K. Reddel[3], Michael J. Abramson[1,2], Cindy Thamrin[3,4] & Mark Hew[1,2,4]

Inhaled corticosteroids (ICS) suppress eosinophilic airway inflammation in asthma, but patients may not adhere to prescribed use. Mean adherence—averaging total doses taken over prescribed—fails to capture many aspects of adherence. Patients with difficult-to-treat asthma underwent electronic monitoring of ICS, with data collected over 50 days. These were used to calculate entropy (H) a measure of irregular inhaler use over this period, defined in terms of transitional probabilities between different levels of adherence, further partitioned into increasing (H_{inc}) or decreasing (H_{dec}) adherence. Mean adherence, time between actuations (Gap_{max}), and cumulative time- and dose-based variability (area-under-the-curve) were measured. Associations between adherence metrics and 6-month asthma status and attacks were assessed. Only H and H_{dec} were associated with poor baseline status and 6-month outcomes: H and H_{dec} correlated negatively with baseline quality of life (H:Spearman $r_S = -0{\cdot}330$, $p = 0{\cdot}019$, H_{dec}:$r_S = -0{\cdot}385$, $p = 0{\cdot}006$) and symptom control (H:$r_S = -0{\cdot}288$, $p = 0{\cdot}041$, H_{dec}: $r_S = -0{\cdot}351$, $p = 0{\cdot}012$). H was associated with subsequent asthma attacks requiring hospitalisation (Wilcoxon Z-statistic $= -2.34$, $p = 0{\cdot}019$), and H_{dec} with subsequent asthma attacks of other severities. Significant associations were maintained in multivariable analyses, except when adjusted for blood eosinophils. Entropy analysis may provide insight into adherence behavior, and guide assessment and improvement of adherence in uncontrolled asthma.

Abbreviations

ACT	Asthma control test
AQLQ	Asthma quality of life questionnaire
AUC	Area under the curve
EMD	Electronic monitoring device
FEV_1	Forced expiratory volume in one second
FeNO	Fraction of exhaled nitric oxide
FVC	Forced vital capacity
GP	General practitioner
H	Entropy
H_{dec}	Decreasing entropy states
H_{inc}	Increasing entropy states
ICS	Inhaled corticosteroids
IgE	Immunoglobulin E
OCS	Oral corticosteroids
PT mean	Conventional mean adherence
SABA	Short acting bronchodilator

In asthma, regular inhaled corticosteroid (ICS) controller use suppresses eosinophilic airway inflammation and reduces airway hyperresponsiveness, reducing symptoms and protecting patients from potentially life-threatening

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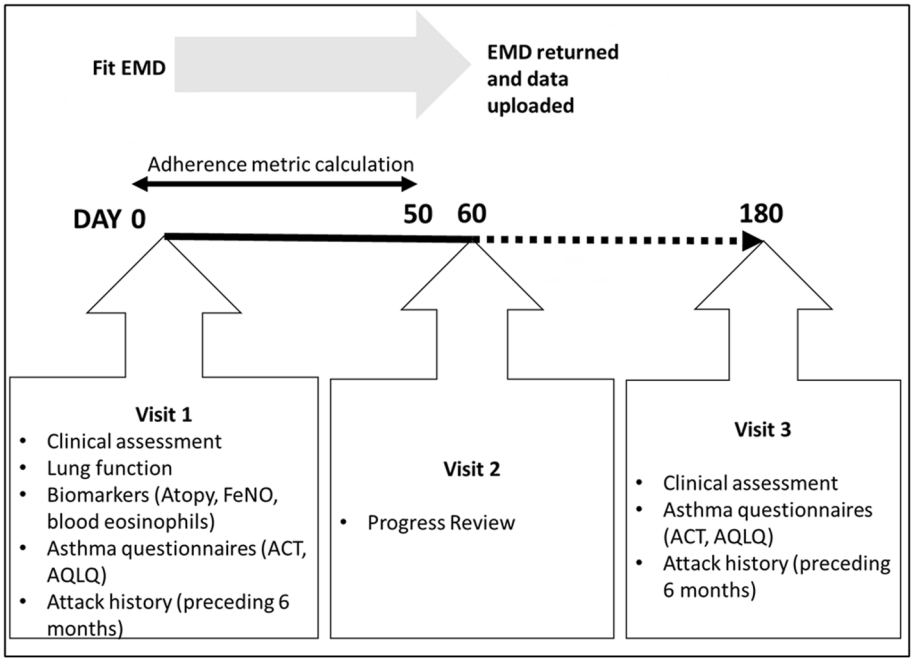

Figure 1. Assessment protocol timeline, visits and clinical measures. *EMD* electronic monitoring device, *ACT* asthma control test, *AQLQ* asthma quality of life. (Microsoft Powerpoint, version 2101, https://office.live.com/start/powerpoint.aspx).

attacks[1,2]. Asthma that remains uncontrolled despite the use of high-dose ICS-based controller is regarded as 'difficult-to-treat', and presents a complex clinical challenge[3].

Medication adherence describes the extent to which patients use medication as prescribed[4,5]. In difficult-to-treat asthma, patients frequently deviate from prescribed use[6,7]. This can be tracked objectively by attaching electronic monitoring devices (EMDs) to a patient's inhaler, recording the date and time of each actuation[8]. In future, electronic monitors may be routinely integrated into inhalers during manufacture[9].

Clarifying the interplay between poor adherence and adverse outcomes could help improve adherence and enhance patient health. So far, work has focused on time-averaged metrics, typically mean adherence (total doses taken/total doses prescribed) and it has been difficult to demonstrate a relationship between asthma outcomes and mean adherence[10,11]. This averaged metric fails to capture potentially important variations in medication-taking behaviour, e.g. a mean adherence rate of 50% cannot distinguish between one patient consistently taking half the prescribed dose daily and another taking the full prescribed dose, but for only half the required period. Other metrics do take into account either the interval between doses, or the time above a minimum dose threshold, and some have shown a relationship to attack rates in airways diseases[10,12]. However, these strategies still only represent summative time-averaged metrics, and do not describe day-to-day deviations from regular prescribed usage.

We designed adherence metrics to capture via EMD the extent to which patients with difficult-to-treat asthma deviate from regular controller usage, by measuring the *entropy*—irregularity, or disorder—with which daily medication doses are taken. The concept of entropy is derived from information theory where it is used to quantify the 'information' in a process. Entropy has been previously applied to respiratory symptoms[13], breathing patterns, and lung function[14]. We examined whether these entropy measures of adherence related to specific patient characteristics or predicted subsequent asthma-related clinical outcomes. For comparison, we also measured conventional mean adherence, time- and dose-based variability (using additional metrics reflecting missed days and incomplete doses respectively), and the duration of gaps in which patients completely forwent medication.

We hypothesised that the degree of *irregularity* of ICS controller usage may be more relevant in difficult-to-treat asthma, and better predict poor outcomes. Highly disordered medication-taking behaviour may place patients at higher clinical risk and may be associated with poorer outcomes.

Methods

Study participants. Our tertiary centre receives referrals of adults with difficult-to-treat asthma from specialists in secondary care[17]. Patients underwent multidisciplinary assessment according to a pre-specified protocol over three visits over six months—previously reported in detail[18–22]—to confirm asthma diagnosis, address comorbidities, and optimise treatment (Fig. 1).

Inhaler technique was reviewed and optimised.

Electronic devices. A compatible EMD (Adherium, Auckland, New Zealand) was fitted to the patient's ICS-containing controller inhaler at visit one (day 0) and EMD-collected ICS data were uploaded at visit two

(approximately day 60, Fig. 1). Outcomes were assessed at visit 3 (~ 180 days). EMDs were available for budesonide/formoterol (Turbuhaler and Rapihaler) and fluticasone proprionate/salmeterol (metered dose inhaler and Accuhaler). To allow uninterrupted monitoring, participants were instructed how to move the EMD if they were to change their inhaler. Audiovisual reminders were not activated during the study period but participants could access 7-day EMD data on their devices.

Clinical outcomes. Evaluation included the Asthma Control Test[23] and Asthma Quality of Life Questionnaire[24] (with permission) at baseline (visit one) and 6 months (visit three) (Fig. 1). Patients were asked to recall the number of attacks in the 6 months prior to visit one, and again, in the 6-month period prior to visit three. Attacks were then confirmed by medical and prescription records where possible. Attacks were also categorised by severity, defined by worsening asthma symptoms requiring: a visit to the general practitioner (least severe); a course (or an increased dose) of oral corticosteroids (OCS; more severe); or hospitalisation (most severe). It was also noted if hospitalization required intensive care admission. Frequency of short acting bronchodilator use over the past four weeks prior to visit one were self-reported and recorded in terms of days and nights per week, as well as number of puffs per day and night.

Patients who completed three visits between August 2015 and February 2018 were eligible for study inclusion. All study protocols and data analysis were approved by the Alfred Health Ethics Committee (285/15) and the Monash University Human Research Ethics Committee (MUHREC). As data were collected as part of routine clinical care and audit, the requirement for signed informed consent was waived by the Alfred Health Ethics Committee. All methods were carried out in accordance with relevant guidelines and regulations as governed by the Australian Health Practitioner Regulation Agency (AHPRA).

Adherence metrics. Metrics were quantified using Python (Python Software Foundation, version 3·6). For standardisation, the first 50 days of available data were extracted for each patient (Fig. 1); this excluded any days with missing data (defined as days when inhaler was not attached—logged by the device as distinct to zero adherence). As the EMD was returned at visit two, adherence data were not available to day 180. Last observation carried forward was not performed to minimise the risk of introducing bias into the adherence metrics, particularly entropy.

Entropy (H), a measure of disorder, was adapted to the adherence data to reflect the various ways in which the patients changed their ICS-taking behavior from day to day. In information theory, a 'Markov chain' can be used to describe the sequence of occurrences of certain 'states' and the probabilities of transitioning from one state to another given the previous state (e.g. the appearance of specific sequences of letters in a message). H is then used to quantify the complexity of the information, in terms of the transitional probabilities between states, for all possible states observed. Here, we classified adherence into different levels, which are analogous to the different states of a Markov chain, similar to an approach previously applied to respiratory symptoms[13]. Given an adherence time series x, where x is the dose taken/prescribed dose expressed as a percentage, we obtained a state-based series by mapping each element of x_i to the state space S = {1,2,3,4,5,6,7} as follows:

State, si	Dose range
1	$x_i = 0\%$
2	$0 < x_i \leq 50\%$
3	$50\% < x_i < 100\%$
4	$x_i = 100\%$
5	$100 < x_i \leq 200\%$
6	$200 < x_i \leq 300\%$
7	$x_i > 300\%$

We then computed the 7 × 7 transitional probability matrix, which comprised probabilities $P_{i,j}$ denoting the probability of transitioning from state *j*, given an initial state *i*, for every combination of states *i,j*. The entropy (H) of the system was determined as $H = \sum_{i,j=0}^{N-1} P_{i,j}(-\ln(P_{i,j}))$, representing the disorder of transitions between daily dose states. An example of H calculation is shown in Fig. 2.

We further partitioned the probability matrix into "increasing" (H_{inc}) or "decreasing" (H_{dec}) adherence, by only considering transitions that moved from lower to higher adherence states on the next available day, or vice versa, respectively, i.e. splitting the transitional probabilities along the diagonal of the matrix. H_{inc} was determined as $\sum_{i,j=0}^{N-1} P_{i,j}(-\ln(P_{i,j}))$, where $i < j$. Similarly, H_{dec} was defined as $\sum_{i,j=0}^{N-1} P_{i,j}(-\ln(P_{i,j}))$, where $i > j$. Thus, while H represents the day to day changes in adherence levels in general, H_{inc} represents the different ways in which a patient may increase their adherence level, and conversely H_{dec} the different ways in which decreases in adherence may occur.

Figure 3 illustrates how different adherence time series with the same conventional mean adherence (PT_{mean}, see below) may vary in H_{inc} and H_{dec}.

Conventional *mean adherence* was described as PT_{mean}, expressed as a percentage of the prescribed number of puffs per day. This was capped at 100%—all higher daily instances were converted to 100% to allow comparison of the mean ($PT_{mean,cap}$) with other published studies. PT_{SD} and PT_{CV} represented the standard deviation and coefficient of variation of PT_{mean} respectively, based on uncapped data to capture the full variability in adherence.

Area under the curve (AUC) measures were inspired by methods previously described[10] to investigate both time- and dose-based adherence variability. In brief, perfect time adherence was first defined as medication taken daily (over the first 50 days), regardless of dosage. The time adherence curve was then defined as the cumulative

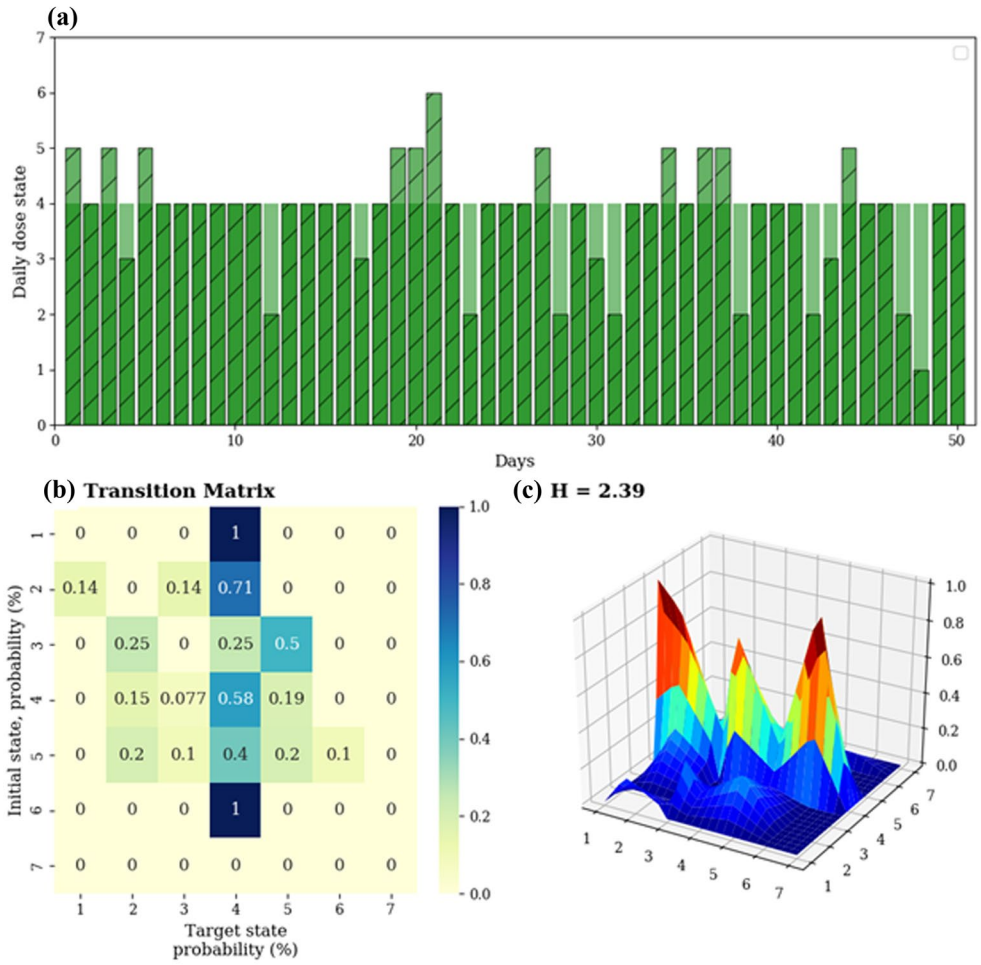

Figure 2. Calculation of entropy. Panel (**a**) shows a perfectly-adherent time series (green) in the background comprising 100% of prescribed puffs for 100% of the time, and an example patient time series (blue) overlaid atop the perfectly-adherent series, with instances of under- and over-adherence, both mapped to the state-based series. Panel (**b**) displays the corresponding transitional probability matrix, while panel (**c**) allows us to visualise the same matrix (and the "disorder") in a 3-dimensional graph. The entropy of the transitional probability matrix is then calculated as $\sum_{i,j=0}^{N-1} P_{i,j}(-\ln(P_{i,j}))$. (Python Software Foundation, version 3·6 http://www.python.org).

sum of every day when medication was taken. Thus, perfect time adherence corresponded to a straight line with an area under the curve normalised to 100%. The time-based AUC (T-AUC) for an individual patient was taken as the difference between their time adherence curve and the perfect curve, expressed as a percentage deviation from 100%. In this way, the T-AUC described how consistently the patient took any medication over time. The use of the cumulative sum meant that earlier and/or larger gaps have greater effects on the T-AUC. Similarly, the dose-based area AUC (D-AUC) described the cumulative deviation from the patient's total prescribed dose over the same 50-day period. We also multiplied the time-based deviation and the dose-based adherence for each day, to construct a composite curve. The Prod-AUC was then calculated as the cumulative deviation from the product of the perfect time x dose curves. This metric thus reflects adherence behavior in terms of both time and doses taken over the given time period.

The Gap_{max} metric described the maximum length of gaps between days when medication was last taken, regardless of number of puffs within a day, during the 50-day period.

Statistical analysis. Relationships between adherence metrics and clinical characteristics (at baseline) and asthma outcomes (at/over six months) were examined using Spearman rank correlation (r_s) for continuous variables, and Wilcoxon rank sum or Kruskal–Wallis tests for comparisons between 2 groups or > 2 groups, respectively. Multivariable regression was performed to confirm if any significant associations between adherence metrics and clinical characteristics and asthma outcomes identified from univariate analyses were still independent predictors after adjusting for the potential confounders of age, sex, baseline eosinophils and baseline lung function. Adjustment for baseline asthma questionnaire scores (AQLQ and ACT) was undertaken where the respective asthma questionnaire score was the outcome.

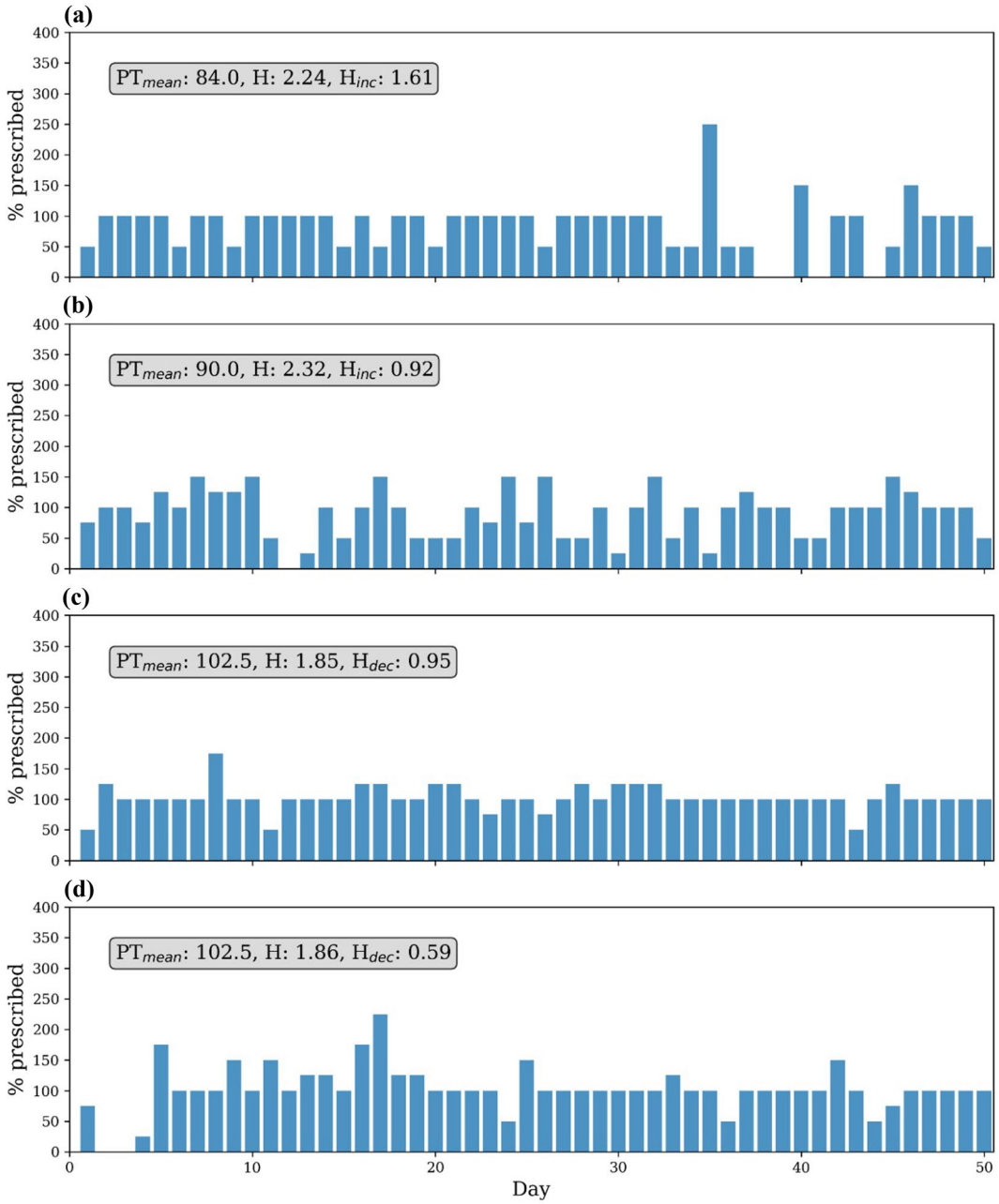

Figure 3. Sample adherence time series from 4 different patients over the study period. All patients had 'good adherence' as defined by mean adherence PT_{mean} (note not capped at 100% for the purpose of demonstrating variability), however with different increasing and decreasing entropy (H_{inc} and H_{dec}) measures, which better reflect the variability in patient inhaler adherence behaviour. Panel (**a**) demonstrates a patient who took their inhaled controller on average 84% of prescribed doses with calculated entropy 2.24 and increasing entropy 1.61. Panel (**b**) demonstrates a second patient who took their controller 90% of prescribed doses, with calculated entropy of 2.32 and increasing entropy of 0.92. Panel (**c**) demonstrates a third patient who took their controller 102.5% of prescribe doses, with calculated entropy of 1.85 and decreasing entropy of 0.95. Panel (**d**) demonstrates a fourth patient who took their controller 102.5% of prescribed doses, with calculated entropy of 1.86 and decreasing entropy of 0.59. (Python Software Foundation, version 3·6 http://www.python.org).

Statistical analysis was undertaken in R version 3·3[25]. Descriptive statistics are presented as proportions for categorical variables, means and standard deviations for normally distributed continuous variables, or medians and interquartile ranges otherwise.

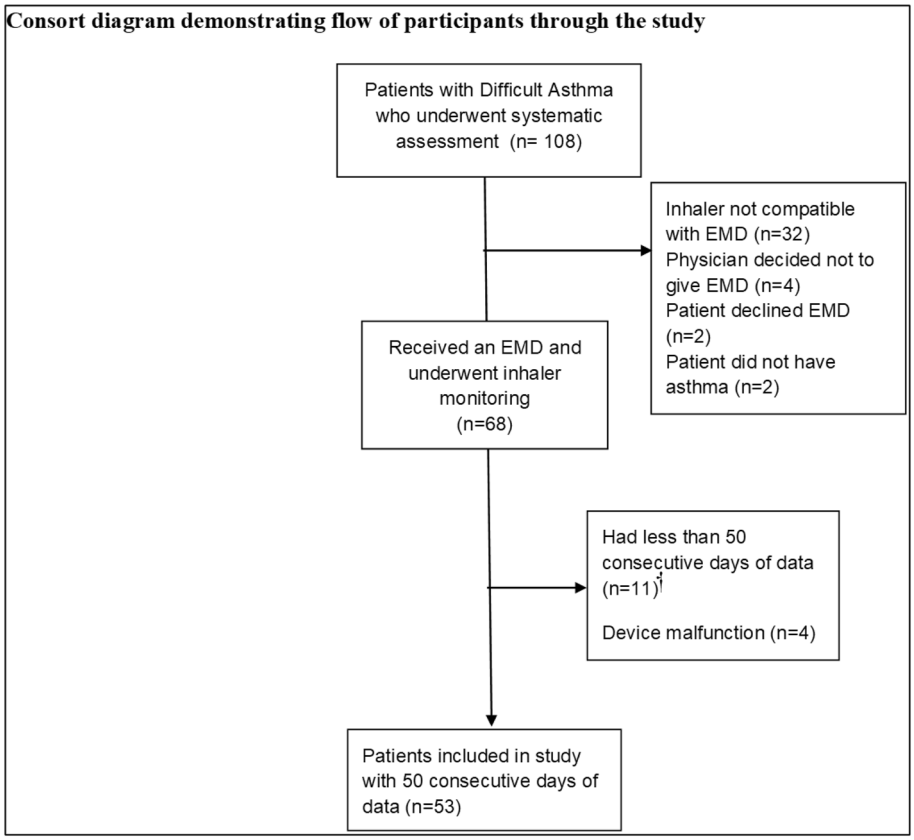

Figure 4. Consort diagram demonstrating flow of participants through the study. *EMD* electronic monitoring device. †Due to EMD detachment. (Microsoft Powerpoint, version 2101, https://office.live.com/start/powerpoint.aspx).

Results

Participants. Systematic assessment was undertaken by 108 patients. Forty (37%) did not receive a monitoring device: four (3.7%) from physician choice, two (1.9%) declined, two (1.9%) did not have asthma (and were diagnosed with vocal cord dysfunction), and 32 (29%) had inhalers with no compatible EMDs available in Australia. Among 68 patients who underwent inhaler monitoring, 11 (16.2%) devices had less than 50 days of data due to device detachment, and 4 (5.9%) devices malfunctioned (Fig. 4). These patients were excluded from the analysis. Of the 11 devices with missing data, the mean number of days monitored was 37, (SD 69, range 3–220 days).

Data were analysed from 53 patients (Table 1).

Adherence metrics and baseline clinical characteristics. Summary statistics of adherence metrics calculated for the first 50 days in all patients are reported in supplemental Table S1. No adherence metric was related to age, sex or baseline lung function on correlation testing (Supplemental Table S2). $PT_{mean,cap}$ correlated with baseline asthma quality of life as measured by AQLQ (Spearman correlation, $r_S = 0{\cdot}284$, $p = 0{\cdot}046$). Large gaps in inhaler use (Gap_{max}) and lower T-AUC were associated with a greater likelihood of previous intensive care or hospitalisation for an asthma attack in the six-month period prior to visit one (Wilcoxon rank sum test), Gap_{max}: $Z = -2.068$, $p = 0{\cdot}039$ and $Z = -2.08$, $p = 0{\cdot}037$ respectively, T-AUC: $Z = -2.065$, $p = 0{\cdot}039$ and $Z = -2.042$, $p = 0{\cdot}041$ respectively).

Regarding entropy, higher H correlated negatively with baseline AQLQ and ACT scores ($r_S = -0{\cdot}330$, $p = 0{\cdot}019$ and $r_S = -0{\cdot}288$, $p = 0{\cdot}041$ respectively). Higher H_{dec} similarly correlated negatively with baseline AQLQ and ACT ($r_S = -0{\cdot}385$, $p = 0{\cdot}006$ and $r_S = -0{\cdot}351$, $p = 0{\cdot}012$ respectively), and was further associated with higher SABA reliever use in terms of puffs and days per week ($r_S = 0{\cdot}318$, $p = 0{\cdot}02$ and $r_S = 0{\cdot}286$, $p = 0{\cdot}04$ respectively).

The relationships between entropy measures (H and H_{dec}) and baseline ACT and reliever use remained significant following multivariable regression models adjusting for age, sex, baseline eosinophil count and baseline FEV, while all other measures did not (Table 2).

Adherence metrics and subsequent outcomes at six months. Among all adherence metrics, only measures of entropy, measured in the first 50 days, were associated with asthma outcomes at six months (Supplemental Table S3). Higher H was associated with more asthma attacks requiring hospitalisation over six months

	Total n = 53
Demographics	
Age years, mean (range, SD)	51 (19–77, 15)
Gender Female, n (%)	29 (55)
Body mass index kg/m^2, mean (SD)	32 (8)
Smoking status	
Never	33 (62.3)
Ex-smoker	17 (32.1)
Current smoker	2 (3.8)
Asthma medications, n(%)	
Short acting muscarinic antagonist	4 (7.5)
Long acting beta agonist	1 (1.9%)
Inhaled corticosteroid	20 (37.7%)
Inhaled corticosteroid/long acting beta agonist combination	52 (98.1%)
Leukotriene receptor antagonist	10 (18.9%)
Long acting muscarinic antagonist	19 (35.8%)
Oral corticosteroids	10 (18.9%)
Theophylline	2 (3.8%)
Omalizumab (anti-IgE monoclonal antibody)	2 (3.8%)
Total number asthma medications, mean, range (SD)	2.3, 1–6 (1.2)
Total daily ICS dose mcg, mean, range (SD)	969, 200–2000 (475)
Asthma severity	
Pre-bronchodilator FEV_1% predicted, mean (SD)	64 (21)
FEV_1/FVC ratio	61 (16)
ACT score at visit one, median (IQR) (23)	11 (9–16·5)
AQLQ score at visit one, mean (SD) (24)	4 (1·2)
On high dose inhaled corticosteroids*, n(%)	44 (83)
Asthma attack rate	
Baseline attack number in the six months prior to visit one (mean, SD)	
Requiring oral corticosteroids	2.5 (2)
Requiring GP visit	2.4 (3.7)
Requiring ED presentation	0.8 (1.3)
Requiring hospital admission	0.4 (1)
Attack rate in the six months prior to visit three (mean, SD)	
Requiring oral corticosteroids	1.7 (2.9)
Requiring GP visit	2 (5.8)
Requiring ED presentation	0.4 (1)
Requiring hospital admission	0.3 (0.8)
Asthma phenotype	
FeNO result ppb, mean (range, SD)	35 (5–137, 32)
IgE kU/L, mean (range, SD)	528 (4–4304, 913)
Atopic (positive skin prick test or serum specific IgE to commonly tested aeroallergens), n(%)	37 (70)
Blood eosinophils × 10^9/L, mean (range, SD)	0·37 (0–1·18, 0·31)

Table 1. Baseline patient characteristics. *FEV_1*.Forced expiratory volume in one second, *FVC* forced vital capacity, *ACT* Asthma Control Test (scores range from 5 (poor asthma control) to 25 (complete asthma control), scores > 19 indicate well controlled asthma), *AQLQ* asthma quality of life questionnaire (out of 7, high score indicating better quality of life). *FeNO* fraction of expired nitric oxide, *IgE* immunoglobulin E, *GP* General practitioner, *ED* emergency department. *Fluticasone propionate equivalent ≥ 500mcg daily.

prior to visit three (Z = − 2.34, p = 0·019, Fig. 5a).Higher H_{dec} was associated with more asthma attacks over the six months prior to visit three, requiring a visit to a general practitioner (Z = − 2.43, p = 0·015), oral corticosteroids (Z = − 2.508, p = 0·012), or hospitalisation (Z = − 2.07, p = 0·038, Fig. 5b–d). (All comparisons performed by Wilcoxon Rank Sums Test).

For regression analysis, it was only possible to adjust for one confounder at a time, due to the amount of patient data available at 6 months. Relationships between H and asthma attacks requiring hospitalisation remained significant regardless of adjustment for age, sex and baseline FEV_1 (Table 3). Similarly, relationships between H_{dec} and attacks requiring oral corticosteroids or general practitioner visits also remained significant with these adjustments (Table 3) and also when adjusted for baseline number of attacks in the six months prior

Baseline measures (adjusted for age, sex, peripheral blood eosinophils and FEV_1)			
Adherence metric	**Baseline measure**	**Coefficient [SE]**	**p value**
Mean adherence ($PT_{mean,cap}$)	AQLQ	0·19 [0.20]	0·36
Entropy (H)	ACT	**−0·49 [0·17]**	**0·008**
	AQLQ	−0·29 [0·15]	0·065
Decreasing Entropy (H_{dec})	ACT	**−0·51 [0·21]**	**0·026**
	AQLQ	−0·35 [0·19]	0·068
	Reliever use, puffs per week	**0·60 [0·28]**	**0·04**

Table 2. Multivariable analysis relating adherence metrics to baseline clinical characteristics. Adherence metrics and baseline measures reported here are those which showed significant associations in univariate analyses. *AQLQ* Asthma quality of life questionnaire, *ACT* asthma control test.

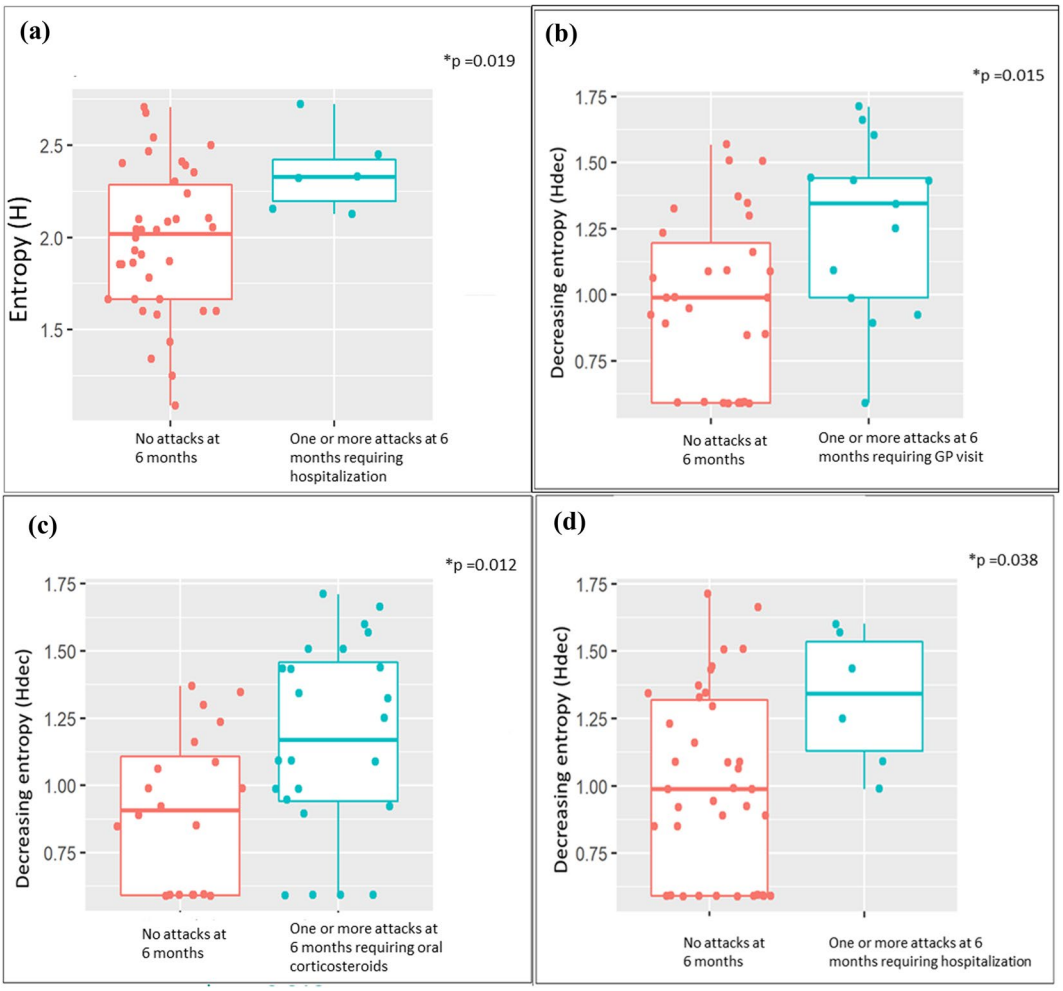

Figure 5. Entropy metrics (over day 0–50) predict asthma outcomes (over days 0–180). Panel (**a**): Entropy (H) and attacks requiring hospitalisation. Panels (**b**–**d**): Decreasing entropy (H_{dec}) and attacks requiring general practitioner (GP) visit, oral corticosteroids or hospitalisation respectively. The boxes depict the 25th, 50th, and 75th percentiles while the whiskers depict the minimum and maximum values in the data. The individual data points are also shown as dots. (R version 3·3 https://www.R-project.org/).

to visit one requiring oral corticosteroids (0.92, SE 0.38, p = 0.017), GP visit (1.06, SE 0.45, p = 0.018) or hospitalization (1.39, SE 0.68 p = 0.041). (Spearman coefficients reported).

However, after adjustment for baseline eosinophil count, the relationships between H or H_{dec} and attacks at six months were no longer significant. Further examination showed that H (but not H_{dec}) was correlated with baseline eosinophil count ($r_S = 0{\cdot}352$, $p = 0{\cdot}045$) suggesting collinearity between baseline eosinophils and H.

Six-month outcomes (adjusted for age, sex, peripheral blood eosinophils and FEV_1)					
Adherence metric	Outcome measure at 6 months	*Coefficient [SE] (p value) when adjusted for*			
		Age	Sex	Blood Eosinophils	FEV_1
Entropy (H)	Attacks requiring hospitalisation	**1·35 [0·68] (p = 0·046)**	**1·34 [0·68] (p = 0·047)**	2·37 [1·50] (p = 0·113)	**1·4 [0·69] (p = 0·044)**
Decreasing Entropy (H_{dec})	Attacks requiring GP visit	**0·98 [0·49] (p = 0·045)**	**0·98 [0·43] (p = 0·021)**	0·91 [0·48] (p = 0·059)	**1·03 [0·43] (p = 0·017)**
	Attacks requiring oral corticosteroids	**0·83 [0·39] (p = 0·034)**	**0·91 [0·38] (p = 0·017)**	0·50 [0·46] (p = 0·284)	**0·99 [0·40] (p = 0·014)**
	Attacks requiring hospitalisation	1·11 [0·60] (p = 0·064)	1·07 [0·59] (p = 0·068)	0·82 [0·69] (p = 0·231)	1·10 [0·58] (p = 0·058)

Table 3. Multivariable analysis relating adherence metrics to 6 month outcomes. *GP* general practitioner. Adherence metrics and outcome measures reported here are those which showed significant associations in univariate analyses.

Discussion

With increasing emphasis on inhaler adherence monitoring in airways diseases, particularly in the era of biologic therapies for severe asthma, there is a pressing need to identify the optimal metrics with which to measure inhaled controller adherence[7,26,27]. We showed that disordered controller use in difficult-to-treat asthma—as reflected by entropy analysis—reflected poor baseline asthma control and were associated with subsequent attacks of any severity. This could potentially be mediated through unchecked eosinophilic inflammation.

Entropy measures have previously been used to describe respiratory symptoms and breathing patterns, with higher entropy associated with adverse outcomes[13,28]. We designed entropy measures (H, H_{dec} and H_{inc}) to measure the irregularity of day-to-day dose-taking behaviour, analogous to the original use of H in information theory to quantify the complexity in strings of text[29]. We used it to describe the diversity in patterns in observed transitions in adherence, choosing to also examine irregularity or diversity in increases and decreases in adherence, as they may be clinically relevant. In considering all (or a subset in the case of H_{dec} and H_{inc}) of the elements in the transitional probability matrix, our method of calculating H differs from that of Usemann et al., where entropy was calculated from rows of elements and then averaged[13]. Nevertheless, H calculated using our method was highly correlated with their method when applied to this dataset ($r = 0{\cdot}912$, $p < 0{\cdot}001$, data available on request).

To accommodate the original study design, we chose a period of 50 days to maximise participants with sufficient data. This proof-of-concept study justifies validation in larger cohorts and the development of more dynamic measures of entropy, similar to our previous work on peak flows to predict attacks[30].

Entropy metrics, specifically in relation to decreasing states (H_{dec}) over a 50-day period, related to worse asthma control and increased short-acting reliever use at baseline. Notably, greater H_{dec} also predicted subsequent risk of attacks of any severity, whether requiring general practitioner visit, increase in oral steroids, or hospitalisation (the latter also predicted by H). That H_{dec}, rather than H_{inc}, has these relationships suggests that irregular drops in adherence may have more clinical impact than over-adherence. These relationships were no longer significant when adjusting for baseline peripheral eosinophil levels. The correlation between H (though not H_{dec}) and peripheral eosinophils suggests that higher baseline eosinophil counts may represent previous poor adherence. We hypothesise that the same pattern of behaviour may then have continued during the period of monitoring, with unchecked eosinophilic inflammation subsequently leading to asthma attacks. Previous studies have demonstrated that peripheral eosinophils are an independent predictor for asthma attacks[31]. Within this small study, baseline blood eosinophils did not predict asthma attacks at 6 months, nor was FeNO related to any adherence measures (supplemental data).

While non-adherence can be intentional due to issues such as mistrust, lack of medication understanding, fixed beliefs and cost, unintentional disordered medication use may also indicate a corresponding degree of chaos in patients' lives. In asthma, poor family routines accompanied diminished inhaler adherence in children[32]. In post-myocardial infarct patients, 'life-chaos'—a highly variable daily routine with an inability to plan and anticipate the future, paralleled poor adherence to cardiac medication[15]. Similar life-chaos among patients with HIV was associated with increased health care use and missed clinic appointments[16]. We speculate that the extent of entropy in controller use in difficult-to-treat asthma may also reflect overall life-chaos. Measurement of entropy in inhaled controller use could be used in the clinic setting to target patients particularly with high H_{dec} for adherence interventions. Such patients may have otherwise been missed if conventional averaged adherence measures were used (Fig. 3). Entropy measures may also prompt the clinician to review the wider social situation of the patient for other indicators of 'life chaos'.

As anticipated, conventional mean adherence ($PT_{mean,cap}$) in our study (following adjustment for potential confounders) was not related to baseline asthma status, nor predicted longitudinal outcomes.

Similarly, neither variability in dosage nor timing metric was associated with clinically important outcomes (Supplemental tables S2 and S3). In a previous analysis of a clinical trial in moderate asthma, the use of AUC-based metrics *did* relate to asthma-related quality of life and lung function by peak flow measurement[10]. Note that our AUC metrics were based upon, but were not directly comparable to previously-published methods[10], which accounted for technique/device errors using a specialised INCA device. Furthermore, our study cohort included consecutive patients drawn from clinical practice.

Our patient population had significant disease with poorly controlled symptoms and high exacerbation rates, despite having previously been assessed by respiratory specialists. We have previously demonstrated that this population still has high non-adherence rates despite specialist intervention. Our results are likely to be representative of difficult-to-treat asthma patients encountered in the 'real world', but may not represent less severe patients. The association of entropy with other behaviour that can affect adherence such as mistrust of medication, financial barriers, and not attending a pharmacy access to refill prescriptions would be worth pursuing with future research.

Limitations

Given the complexity of difficult-to-treat asthma, poor disease control may relate to a wide range of disease and patient factors, e.g. biological severity, corticosteroid insensitivity, multimorbidity, poor self-management skills—all addressed in our clinic's systematic protocol[33–35]. Notwithstanding the presence of such confounding issues, a significant effect of disordered controller use on risk of asthma attack remained detectable. However, it is possible our single-center study had insufficient statistical power from a reduction in data available due to device incompatibility device malfunction, missing data, small sample size and short duration of data collection, to detect weaker associations. We also relied on patient recollection for asthma attack history which could be inaccurate, although these data were verified when available in medical records. We explored a range of metrics, baseline characteristics, and asthma outcomes, so increasing the likelihood of a chance finding. However, the consistent pattern of results and their persistence following adjustment for confounding both support a true result. We only collected adherence data between visit one and two of our study (most consistently for 50 days), and analysed outcomes at day 180 (visit three). It is possible that adherence would have improved beyond 50 days, however we wished to analyse the impact of the patient's initial adherence behaviour on future asthma outcomes. It is likely other aspects of adherence behaviour would add to the predictive power of entropy measures; larger validation datasets would enable further exploration as well as control for other possible confounders in the same model. Future studies could also explore the impact of patient socioeconomic status or device polypharmacy on entropy of inhaled controller use as well as examine aspects of 'life chaos' more qualitatively.

Conclusions

We showed higher irregularity assessed by entropy in controller use of patients with difficult-to-treat asthma, with effects that appear mediated through eosinophilic inflammation, and were associated with an increased risk of future attacks. Entropy may reflect the 'life chaos' experienced by people with difficult-to-treat asthma, a possible target for appropriate intervention. Entropy analysis could guide future approaches to improve adherence and enhance patient health, potentially applicable to other domains of respiratory or other chronic disease.

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Author contributions

The study was conceived by J.L., M.H., J.F. and C.T. Clinical data was collected by J.L. and M.H. Data, statistical analysis and entropy calculations were performed by J.H. and C.T. Data interpretation was performed by all authors. Figures were prepared by J.L., J.H. and C.T. Following input by all authors, the first draft was written by M.H., J.L. and C.T. All authors participated in editing and discussion. Study supervision was by M.H. and C.T.

Competing interests

MJA holds investigator-initiated grants for unrelated research from Pfizer and Boehringer-Ingelheim. He has undertaken an unrelated consultancy for and received assistance with conference attendance from Sanofi. He has also received a speaker's fee from GSK. HKR or her institute has received fees for providing independent medical advice on advisory boards for AstraZeneca, GlaxoSmithKline, Boehringer Ingelheim, GlaxoSmithKline, Novartis and Sanofi/Genzyme, for providing independent medical education at symposia funded by AstraZeneca, Boehringer Ingelheim, GlaxoSmithKline, Mundipharma, Novartis and Teva, and research grants from AstraZeneca, GlaxoSmithKline and Novartis, all unrelated to this research. MH has received grants-in-aid, speaker fees, and fees for serving on the advisory boards of GlaxoSmithKline, AstraZeneca, Novartis, Teva, Sanofi, and Seqirus, all unrelated to the current manuscript, all paid to his institutional employer Alfred Health. JL has received fees for providing unrelated independent medical advice for GlaxoSmithKline and has provided speaker fees for medical education purposes from Boehringer Ingelheim, GlaxoSmithKline and AstraZeneca. HR reports grants and personal fees from AstraZeneca, grants and personal fees from GlaxoSmithKline, personal fees from Merck, grants and personal fees from Novartis, personal fees from Teva, personal fees from Boehringer Ingelheim, personal fees from Sanofi Genzyme, outside the submitted work. CT is a NHMRC Career Development Fellow (Level 1). JL received support through an Australian Government Research Training Program Scholarship. Both funding sources had no role in study design, collection, analysis, interpretation of data, writing of the report of decision to submit this manuscript for publication. JH and JF have no competing interests to disclose.

Additional information

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SUPPLEMENTARY INFORMATION

Table S1. Summary statistics of adherence metrics calculated for the first 50 days in all patients

Adherence metric	Median (25^{th} centile, 75^{th} centile)
$PT_{mean,cap}$, %	88 (66.5, 93)
PT_{mean}, %	92 (72, 101)
PT_{SD}, %	34.5 (25.9, 44.8)
PT_{CV}, %	40.7 (29.4, 57.7)
Gap_{max}, days	1 (0, 4)
Entropy (H)	2.04 (1.66, 2.32)
Increasing Entropy (H_{inc})	1.09 (0.95, 1.16)
Decreasing Entropy (H_{dec})	1.09 (0.59, 1.37)
T-AUC, %	-1.76 (-8.16, 0.00)
D-AUC, %	-7.36 (-26.6, 2.26)
Prod-AUC, %	-9.45 (-34.37, 0.08)

Please see main text for adherence metrics abbreviations

Variable	$PT_{mean,cap}$	PT_{mean}	PT_{SD}	PT_{CV}	Gap_{max}	H	H_{inc}	H_{dec}	T-AUC	D-AUC	Prod-AUC
Sex (male)†	-1.47(0.14)	-1.35(0.18)	-0.49(0.62)	-1.06(0.29)	-0.48(0.63)	-0.61(0.54)	-0.63(0.53)	-0.57(0.57)	-0.53(0.60)	-1.15(0.25)	-1.28(0.20)
Age (years) ††	-0.056(0.69)	-0.072(0.61)	-0.018(0.90)	-0.008(0.96)	0.072(0.61)	-0.074(0.60)	-0.018(0.9)	-0.212(0.13)	-0.039(0.78)	-0.034(0.81)	-0.02(0.89)
FEV_1 (litres) ††	0.13(0.37)	0.19(0.18)	-0.015(0.92)	-0.104(0.46)	-0.077(0.58)	-0.099(0.48)	0.091(0.52)	-0.116(0.41)	0.079(0.57)	0.153(0.27)	0.15(0.29)
Eosinophils ($x10^9$/L) ††	-0.31(0.08)	-0.31(0.08)	-0.036(0.84)	0.24(0.17)	0.22(0.21)	0.352(0.045*)	0.291(0.1)	0.109(0.547)	-0.29(0.1)	-0.25(0.169)	-0.23(0.21)
Reliever use (puffs per week) ††	-0.133(0.35)	-0.213(0.13)	-0.279(0.045*)	-0.169(0.232)	-0.224(0.11)	0.214(0.13)	-0.175(0.22)	0.336(0.015*)	0.20(0.15)	-0.25(0.07)	-0.23(0.11)
ACT score††	0.155(0.28)	0.16(0.26)	0.114(0.42)	0.078(0.59)	0.113(0.43)	-0.288(0.041*)	0.112(0.44)	-0.351(0.012*)	-0.064(0.66)	0.17(0.24)	0.16(0.25)
AQLQ score††	0.284(0.046*)	0.247(0.08)	0.006(0.97)	-0.146(0.31)	-0.068(0.64)	-0.33(0.019*)	-0.012(0.93)	-0.385(0.006**)	0.116(0.422)	0.26(0.07)	0.25(0.08)

Table S2: Baseline Univariate Analyses

†Analysis – Wilcoxon Rank Sums, ††Analysis – Spearman correlation. Z statistic or Spearman correlation coefficient shown, with P values in brackets.

*significant to p<0.05, **significant to p<0.005. Reliever use -daytime average of short acting beta agonist over seven-day period, Eosinophils – peripheral blood sample, FEV_1 – Forced expiratory volume in one second, ACT – asthma control test, AQLQ – asthma quality of life questionnaire

Please see main text for adherence metrics abbreviations

Table S3: 6-month outcomes univariate analyses

Exacerbations in 6 months	**$PT_{mean,cap}$**	**PT_{mean}**	**PT_{SD}**	**PT_{CV}**	**Gap_{max}**	**H**	**H_{inc}**	**H_{dec}**	**T-AUC**	**D-AUC**	**Prod-AUC**
Requiring OCS	-0.08 (0.93)	-0.34 (0.73)	-1.24 (0.22)	-0.81 (0.42)	-1.72 (0.09)	-1.33 (0.18)	-1.46 (0.15)	-2.51 (0.012*)	-1.53 (0.13)	-0.34 (0.73)	-0.13 (0.90)
Requiring GP visit	-0.59 (0.55)	-0.57 (0.57)	-0.03 (0.98)	-0.46 (0.64)	-0.61 (0.54)	-0.58 (0.56)	-0.18 (0.86)	-2.43 (0.015*)	-0.32 (0.75)	-0.57 (0.57)	-0.54 (0.59)
Requiring ED visit	-1.39 (0.17)	-1.81 (0.07)	-0.41 (0.68)	-0.53 (0.59)	-0.08 (0.94)	-1.13 (0.26)	-0.18 (0.86)	-0.71 (0.48)	-0.11 (0.91)	-1.81 (0.07)	-1.87 (0.061)
Requiring hospitalisation	-1.23 (0.22)	-1.56 (0.12)	-0.26 (0.80)	-0.46 (0.64)	-0.07 (0.94)	-2.34 (0.019*)	-0.77 (0.44)	-2.07 (0.038*)	-0.087 (0.93)	-1.52 (0.13)	-1.52 (0.13)

All analyses completed via Wilcoxon Rank Sum. Z statistic shown, with P values in brackets. OCS – oral corticosteroid course or increase in steroid dose from baseline, GP – general practitioner, ED- emergency department. *significant to p<0.05 level

4.2 Summary of findings from Chapter 4

In this study, only entropy measurements reflected poor asthma baseline status and predicted asthma outcomes over the six-month period of the study. In particular, higher entropy (H) was associated with asthma attacks requiring hospitalisation, and higher H_{dec} was associated with asthma attacks requiring oral corticosteroids, a visit to the general practitioner and hospitalisation. These relationships were still seen even after adjustment for possible confounders including age, sex and lung function (as measured by FEV_1). I hypothesised that the use of oral steroids as 'rescue therapy' would be associated with more chaotic inhaled corticosteroid use. The results were consistent with this hypothesis by demonstrating a relationship between oral steroid use and H_{dec}. Therefore if patients have variable exposure to ICS over time but are receiving multiple courses of steroids, their H_{dec} was likely to be high. Collinearity was identified when H was related to peripheral blood eosinophil count, suggesting that effects of entropy on outcome measures may be mediated via unchecked eosinophilic inflammation. This study did not perform subgroup analyses on the subpopulation of patients employing the MART approach (budesonide/formoterol maintenance and reliever therapy). Future studies specifically examining this population would be worthwhile to undertake given the inherent variability with this inhaler strategy to determine if the relationships between entropy and exacerbation rates are replicable in this patient group.

Future studies could also investigate what may underly the higher entropy measures, such as via a focus group of the "high entropy" subset of patients. In my study, we looked at the influence of comorbidities such as substance abuse and anxiety and depression on Entropy, but the small patient numbers meant that a significant association was not identified.

Research in other fields such as bronchiectasis has demonstrated that patients with more severe disease are more likely to be affected by other factors such as fewer transport options to clinic appointments, lower socioeconomic status or being from indigenous populations(145). If barriers to adherence are to be addressed, it would be important to identify these underlying contributing cofactors to entropy. Nevertheless, entropy analysis of inhaled corticosteroid use may be useful to identify patients most at risk of poor outcomes from poor inhaler adherence.

SECTION II: VOCAL CORD DYSFUNCTION AND COUGH HYPERSENSITIVITY IN DIFFICULT ASTHMA

Introduction

This section contains three chapters addressing issues affecting the "middle airway" as contributing comorbidities that complicate the diagnosis and treatment of difficult asthma. Traditionally, airway dysfunction has been separated into conditions of the upper (nose, sinuses and pharynx) and lower (respiratory tract) airways. However, in 2013, Bardin and colleagues called attention to dysfunction of the middle airway (larynx, trachea), a neglected site of pathology which is often not considered by many respiratory physicians when patients present with breathlessness.(146) Asthma in particular is a common coexisting or misdiagnosis for middle airway, or laryngeal dysfunction. In a recent cross-sectional observational study, 87% of severe asthma patients were found to have laryngeal dysfunction affecting respiration, phonation or both.(107)

Laryngeal dysfunction is an umbrella term and includes a spectrum of disease entities including vocal cord dysfunction (VCD), also termed inducible laryngeal obstruction (ILO), chronic cough hypersensitivity syndrome, muscle tension dysphonia and chronic *globus pharyngeus*.

VCD/ILO occurs when vocal folds or supraglottic structures paradoxically adduct during respiration. When misdiagnosed and mistreated as asthma, there can be unnecessary use of escalating doses of inhaled corticosteroids and high health care utilisation.(147) Due to the similarity of presenting symptoms and disparity of effective treatment options, a systematic process to objectively confirm the presence and relative contribution of each diagnosis to an individual patient's clinical presentation becomes essential but has not previously been described in difficult to treat asthma. *Chapter five: Diagnosis of concomitant inducible laryngeal obstruction and asthma* described a multidisciplinary systematic and objective protocol to diagnose the two conditions. Why VCD occurs in difficult-to-treat asthma, and which clinical features best predict its presence are also important unanswered questions. These research questions are addressed in *Chapter six: Paradoxical vocal fold motion in difficult asthma is associated with dysfunctional breathing and preserved lung function.*

Chronic cough is common in laryngeal dysfunction and difficult-to-treat asthma. While pathophysiology of cough may be due to asthma-related airway inflammation and subsequent bronchial reactivity, it has been increasingly recognised that hypersensitivity of the afferent cough neuronal reflexes is a likely contributing factor.(136) The respective prevalence of asthma-related cough compared to laryngeal dysfunction-related cough had not previously been described, nor the clinical features that might help to identify these differences. This knowledge gap was specifically examined in the study presented in *Chapter seven: Laryngeal hypersensitivity in patients undergoing bronchial provocation challenge with mannitol.*

Chapter Five: The Diagnosis of Concomitant VCD and Asthma

5.1 Introduction

This study prospectively evaluated 69 patients who presented with concomitant suspected ILO (VCD) and/or asthma. A multidisciplinary and systematic assessment protocol was followed using stepwise diagnostic procedures to identify paradoxical adductory vocal cord movement and/or variable airflow obstruction. Validated comorbidity and laryngeal dysfunction questionnaire data as well as baseline characteristics and clinical features were also collected. These data were then compared between four patient groups: patients with asthma alone, patients with ILO alone, patients with both asthma and ILO, and patients with neither diagnosis objectively confirmed.

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ORIGINAL ARTICLE

Asthma and Rhinitis

WILEY

Diagnosis of concomitant inducible laryngeal obstruction and asthma

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Summary

Background: Inducible laryngeal obstruction, an induced, inappropriate narrowing of the larynx, leading to symptomatic upper airway obstruction, can coexist with asthma. Accurate classification has been challenging because of overlapping symptoms and the absence of sensitive diagnostic criteria for either condition.

Objective: To evaluate patients with concomitant clinical suspicion for inducible laryngeal obstruction and asthma. We used a multidisciplinary protocol incorporating objective diagnostic criteria to determine whether asthma, inducible laryngeal obstruction, both, or neither diagnosis was present.

Methods: Consecutive patients were prospectively assessed by a laryngologist, speech pathologist and respiratory physician. Inducible laryngeal obstruction was diagnosed by visualizing paradoxical vocal fold motion either at baseline or following mannitol provocation. Asthma was diagnosed by physician assessment with objective variable airflow obstruction. Validated questionnaires for laryngeal dysfunction and relevant comorbidities were administered.

Results: Of 69 patients, 15 had asthma alone, 11 had inducible laryngeal obstruction alone and 14 had neither objectively demonstrated. Twenty-nine patients had both diagnoses. In 19 patients, inducible laryngeal obstruction was only seen following provocation. Among patients with inducible laryngeal obstruction, chest tightness was more frequent with concurrent asthma. Among patients with asthma, stridor was more frequent with concurrent inducible laryngeal obstruction. Cough was more frequently found in asthma alone, whereas difficulty with inspiration and symptoms triggered by psychological stress were more frequently found in inducible laryngeal obstruction alone. Patients with asthma alone had greater airflow obstruction. Relevant comorbidities were frequent (rhinitis in 85%, gastro-oesophageal reflux in 65%), and questionnaire scores for laryngeal dysfunction were abnormal. However, neither comorbidities nor questionnaires differentiated patients with or without inducible laryngeal obstruction.

Conclusions and clinical relevance: In this cohort with suspected inducible laryngeal obstruction and asthma, 42% had objective evidence of both conditions. Clinical

assessment, questionnaire scores and comorbidity burden were not sufficiently discriminatory for diagnosis, highlighting the necessity of objective diagnostic testing.

KEYWORDS
asthma, inducible laryngeal obstruction, larynx, mannitol, paradoxical vocal fold motion

1 | INTRODUCTION

Inducible laryngeal obstruction (ILO) is characterized by recurrent variable airflow obstruction in the larynx. It is an umbrella term for any triggered laryngeal obstruction occurring at either the supraglottic (arytenoid region, epiglottis or false vocal folds) and/or glottic (true vocal folds) level of the larynx and includes the condition also known as paradoxical vocal fold motion disorder (PVFM), or vocal cord dysfunction (VCD).[1,2] Traditionally, the gold standard for diagnosis has been the demonstration on direct laryngoscopy of abnormal (hence paradoxical) adduction of the vocal folds during the respiratory cycle accompanying a symptomatic episode.[3]

Although patients with symptoms and signs suggestive of ILO were described as early as 1885[4] advances in this field may have been hindered in part by a belief that the condition was psychosomatic in origin.[1] More recently, ILO has been considered as part of a spectrum of laryngeal dysfunction, which also includes chronic cough, globus pharyngeus and muscle tension dysphonia. All are thought to share a common pathophysiological pathway of laryngeal hypersensitivity, or "irritable larynx."[5,6]

Confusingly, ILO and asthma share many common symptoms, such as dyspnoea, chest tightness and wheeze. Distinguishing between the two conditions may be challenging and validated diagnostic and treatment algorithms have not yet been established.

Compounding this difficulty, asthma and ILO are not mutually exclusive. A high proportion of patients with difficult-to-treat asthma may have comorbid ILO,[7] with a reported prevalence of ILO in this population of between 30% and 50%.[8,9] Patients with asthma and concurrent ILO are more likely to have poor quality of life and increased healthcare utilization.[10] Importantly, the treatment modalities for asthma and ILO are vastly different.

Our centre provides a difficult asthma service which focuses heavily on comorbidity assessment, including ILO.[11,12] As an extension of this service, we developed a multidisciplinary Middle Airway Clinic to systematically assess and manage patients with suspected concurrent asthma and ILO. We report the clinical characteristics and objectively determined diagnoses of a consecutive series of patients undergoing systematic evaluation.

2 | METHODS

Our centre is a 600 bed tertiary hospital in Melbourne, Australia. The Middle Airway Clinic was established in May 2015 specifically for the diagnosis and management of patients with concurrent suspected diagnoses of inducible laryngeal obstruction and asthma. Patients were referred by specialists, either from the respiratory and allergy units, or from the Ear, Nose and Throat surgical unit. All patients underwent systematic assessment by a laryngologist, speech pathologist and respiratory physician. This report was approved by the Alfred Health Ethics committee (Reference number 37/16).

2.1 | Multidisciplinary assessment

Baseline characteristics and presenting symptoms were documented by the respiratory specialist (RH, TT, JL) using a standardized clinic template. We assessed for the presence of all forms of laryngeal dysfunction: ILO, chronic cough, muscle tension dysphonia and globus pharyngeus. Triggers or "inducers" of symptoms were classified as inhalational (odours or perfume, chemical or cleaning solutions, smoke and exhaust fumes), physiological (exercise) or psychological. Patients completed validated questionnaires for laryngeal dysfunction: Pittsburgh Vocal Cord Dysfunction Index,,[13] VCD-Questionnaire,[14] and the Newcastle Laryngeal Hypersensitivity Questionnaire,[15] as well as the Chemical Sensitivity Scale for Sensory Hyper-reactivity[16] and the Dyspnoea Index.[17] (See Table S1).

Laryngologist evaluation (PP, AR) comprised an ENT history and examination followed by flexible laryngoscopy to assess for paradoxical vocal fold motion, both at rest and following provocation manoeuvres. Any inducible laryngeal obstruction was classified, when possible, by location (supraglottic, glottic or both) and phase of respiratory cycle (inspiratory, expiratory or both) as described in recent consensus statements.[1,2] We also examined for laryngoscopic evidence of exacerbating conditions such as laryngopharyngeal reflux, chronic rhinosinusitis and oral candidiasis. Video stroboscopy was performed when clinically relevant and available.

A comprehensive asthma history was elicited. Variable airflow obstruction was sought in a stepwise fashion based on bronchodilator reversibility, peak flow variability and bronchoprovocation with mannitol. Spirometry was scheduled for all patients (Medgraphics Platinum, MGC Diagnostics, Minnesota, USA). Patients were asked to withhold medications that may affect bronchial hyperresponsiveness as per the Aridol protocol.[18] All inhaled corticosteroids with long acting beta2 agonists were withheld for at least 24 hours. Patients were assessed for allergy and designated atopic if they had at least one wheal $\geq$3 mm on skin prick testing to twenty aeroallergens (Stallergenes-Greer®, Antony, France) or a serum allergen-specific IgE >0.34 kUA/L (ImmunoCap® Abacus ALS, Brisbane, Australia) to at least one of: Ryegrass pollen, house dust mite or *Aspergillus*.

Speech pathologist review (LP, MV) included a comprehensive voice assessment with auditory-perceptual evaluation using the GRBAS (Grade, Roughness, Breathiness, Asthenia and Strain) scale

and where appropriate, measurement of the maximum phonation time (MPT). Posturing, vocal hygiene and swallow were also clinically assessed.

2.2 | Diagnosis of asthma and inducible laryngeal obstruction

A diagnosis of definite ILO was made by the demonstration of any vocal cord adduction during inspiration, or >50% vocal cord adduction on expiration, either at baseline laryngoscopy, or on laryngoscopy following provocation with dry powder mannitol (Aridol™, Pharmaxis, NSW, Australia) as previously described.[19] Inappropriate adduction of the vocal folds in expiration was determined by the observer as more than 50% reduction in the area of the rima glottidis, or laryngeal inlet airspace between the edges of the true vocal cords. If laryngoscopy was performed by respiratory physician, confirmation was sought from blinded laryngologist review. The angle at the anterior commissure from the position of the vocal processes was observed and was also considered positive if there was more than 50% reduction in the angle.

A diagnosis of definite asthma was confirmed by demonstrating variable airflow obstruction based on bronchodilator response (Increase in ≥200 mL and 12% from baseline FEV_1 or FVC),[20] peak flow variability >15%, or positive bronchial provocation challenge (≥15% drop in FEV_1 with cumulative mannitol dose <635 mg).

Although many patients had a clinical history suggestive of ILO and asthma and were eventually treated as such, for the current study analysis, the "ILO" group comprised only patients with objective findings of paradoxical vocal cord movement confirming definite ILO. Similarly the "asthma" group comprised only patients with demonstrable variable airflow obstruction confirming definite asthma.

2.3 | Comorbidity assessment

Patients were assessed for the presence of eight comorbidities: obesity, allergic rhinitis, chronic rhinosinusitis, gastroesophageal reflux disease, obstructive sleep apnoea, anxiety, depression and dysfunctional breathing. Comorbidity diagnosis was assisted by a battery of validated questionnaires (Table S2**). These were the Score for Allergic Rhinitis,[21] Rhinitis Control Assessment Test,[22] Gastroesophageal reflux disease Questionnaire,[23] Hospital Anxiety and Depression Scale,[24] Berlin[25] and Nijmegen questionnaires.[26]

2.4 | Statistical analysis

Data analysis was performed using SPSS version 22 (IBM, Armonk, NY). Categorical variables are presented as percentages (frequency) and continuous variables as mean or median values with standard deviations. Student's *t* tests or one-way ANOVA were performed for comparison of means, and Kruskal-Wallis tests followed by post hoc Mann-Whitney tests were performed for comparison of non-parametrically distributed continuous data. Fisher's exact or *chi*-square tests were performed for comparison of proportions as appropriate.

3 | RESULTS

3.1 | Patients included

Sixty-nine consecutive patients were assessed between 1 May 2015 and 1 February 2017 with the suspicion of co-existing inducible laryngeal obstruction and asthma. Thirty-one patients (45%) were referred from the difficult asthma service, 35 (51%) from general respiratory/allergy clinics and three (5%) from the general ear, nose and throat clinic. Baseline demographics are presented in Table 1.

3.2 | Assessment procedures

All 69 patients underwent clinical assessment. All but one patient (who declined) completed the questionnaire battery. All but one (who declined) underwent spirometry. Sixty-seven patients underwent flexible nasoendoscopy ± stroboscopy, of whom 42 patients had nasoendoscopy performed as part of a mannitol challenge test. Two patients did not undergo nasoendoscopy; one patient declined

TABLE 1 Baseline demographics of patients

Age, mean (SD) y	47 (15), range 18-72
Gender, n (%)	
Female	55 (80%)
BMI, mean (SD), kg/m^2	29.6 (5.6)
BMI ≥30, n (%)	28 (40%)
Smoking status, n (%)	
Never	45 (65%)
Ex-smoker	22 (32%)
Current smoker	2 (3%)
Pre bronchodilator FEV_1(%predicted), mean (SD)	81.7% (21)
Pre bronchodilator FVC (%predicted), mean (SD)	87.8% (19)
Pre bronchodilator FER, mean (SD)	73.4% (12.5)
Pre bronchodilator FER <70% (airflow obstruction), n (%)	24 (35)
Medication use, n (%)	
Inhaled corticosteroids	50 (73%)
Intranasal corticosteroids	38 (55%)
Antihistamines	24 (35%)
Proton pump inhibitors	31 (45%)
Atopic[a], n (%)	36 (52%)
Occupation, n (%)	
Unemployed	15 (22%)
Cleaner	7 (12.3%)
Healthcare professional	13 (19%)
Professional voice user, including teachers	12 (17.4%)

FEV_1, Forced expiratory volume in one-second; FVC, forced expiratory volume; FER, forced expiratory ratio.
[a]Defined in text.

the procedure while the other had severe airflow obstruction and a clinical history inconsistent with ILO and was therefore not tested.

Forty of 69 patients (58%) had definite ILO with objectively visualized paradoxical vocal fold motion. Of these patients, 18 (45%) were diagnosed at baseline laryngoscopy, 19 (47.5%) following mannitol challenge, and three (7.5%) underwent laryngoscopy under both conditions. Of the 40 patients with ILO, 10 (24.4%) patients had inspiratory, 14 (34.1%) had expiratory and 10 (24.4%) had both inspiratory and expiratory ILO identified. Only two patients (5%) had obstruction documented at the level of the supraglottis. Six patients with laryngologist-visualized ILO did not have the type of ILO specified.

In patients with ILO, the median time to diagnosis was 5.5 years. Eleven patients (27.5%) described an incident at onset, including nine triggered by respiratory infection (22.5%), one (2.5%) with environmental irritant exposure and one reportedly triggered following a general anaesthetic. Over half of all patients had presented to the emergency department with symptoms (62.5%). Inhaled odours (55%), exercise (57.5%) and psychological stress (57.5%) were common triggers for symptoms. An additional third of patients described triggers in their workplace including: cleaning products, chlorine, chalk dust, dust, singing, voice use and work-related stress.

Forty-four of 69 patients (64%) were diagnosed with definite asthma, with proven variable airflow obstruction. Of these, 19 (43%) patients had reversibility on spirometry, 6 (13.6%) patients had peak flow variability and 19 (43%) had positive bronchial provocation challenges.

3.3 | Co-existence of ILO and asthma

The total numbers of patients with or without ILO and asthma are shown in Table 2.

3.3.1 | Patients without asthma or ILO (A−I−)

Of the 14 patients with neither objectively demonstrable ILO nor asthma, one had globus pharyngeus and vocal cord paresis, two had chronic cough with laryngeal hypersensitivity, one had post-nasal drip due to chronic rhinitis and one had laryngeal hypersensitivity associated with recurrent upper airway angioedema. The remaining nine patients were still thought to have either probable ILO or asthma based on clinical assessment, but neither could be proven objectively. Of these 14 patients, nine were on inhaled corticosteroids for suspected asthma. Following evaluation, six of these nine patients had their inhaled corticosteroids ceased and an additional patient had the dose of inhaled corticosteroids reduced. Two patients were thought to have well-controlled asthma and thus were continued on their prescribed treatment.

TABLE 2 Asthma and ILO diagnoses

		ILO	
		No	Yes
Asthma	No	14 (20.3%)	11 (15.9%)
	Yes	15 (21.7%)	**29 (42%)**

ILO, inducible laryngeal obstruction.
Bold - patients diagnosed with concomitant asthma and ILO.

3.3.2 | Patients with asthma only (A+I-)

Of the 15 patients with asthma but not ILO, five were thought to have symptoms attributable only to asthma. Another six patients had additional contributors to symptoms; two had dysfunctional breathing, one had angioedema and three had an irritable larynx in the context of high-dose inhaled corticosteroid treatment for asthma. The remaining four patients were treated for symptoms of ILO based on clinical assessment. As a direct result of the clinic evaluation, three patients were commenced on inhaled corticosteroids for uncontrolled asthma, while the other patients had their existing asthma treatment adjusted as per treatment guidelines.

3.3.3 | Patients with ILO only (A−I+)

Eleven patients were classified as having ILO only. Six of these patients had been on high dose inhaled corticosteroids or combination therapy for asthma. One patient was found to have COPD. The remaining four patients were using intermittent short acting bronchodilators. Following clinical evaluation, three of the six patients had their inhaled corticosteroids ceased and the remaining three had their cumulative steroid dose reduced with aim to wean. The remaining four patients were encouraged to use speech pathology techniques for management of their symptoms rather than short acting bronchodilators.

3.3.4 | Patients with both asthma and ILO (A+I+)

Of the 29 patients with both ILO and asthma, the managing clinician's impression was that ILO predominantly contributed to symptoms in 41%, while asthma predominantly contributed to symptoms in 24%; dual contribution occurred in 14% (Figure 1). Management of these patients included speech therapy and/or titration of asthma treatment according to recognized guidelines (GINA).

3.3.5 | Comparison between groups

When patients with both ILO and asthma (A+I+) were compared to patients with only ILO (A−I+), chest tightness was significantly more common in patients with both ILO and asthma ($P = .01$) than in those with ILO alone. Forced expiratory ratio was also significantly higher in patients with ILO alone when compared to patients with both asthma and ILO ($P = .009$) (Figures 2 and 3).

There was a statistically significant difference in forced expiratory ratio between the four different patient groups (Kruskal–Wallis test χ^2 (3) = 21.17, $P < .0001$), with a mean rank FER of 46.5 for patients without A−I−, 30.19 for A+I+, 48.68 for A−I+ and 19.2 for A+I−. Post hoc analyses demonstrated that the significant differences were between patients with and without asthma, regardless of the presence of ILO (Figure 3).

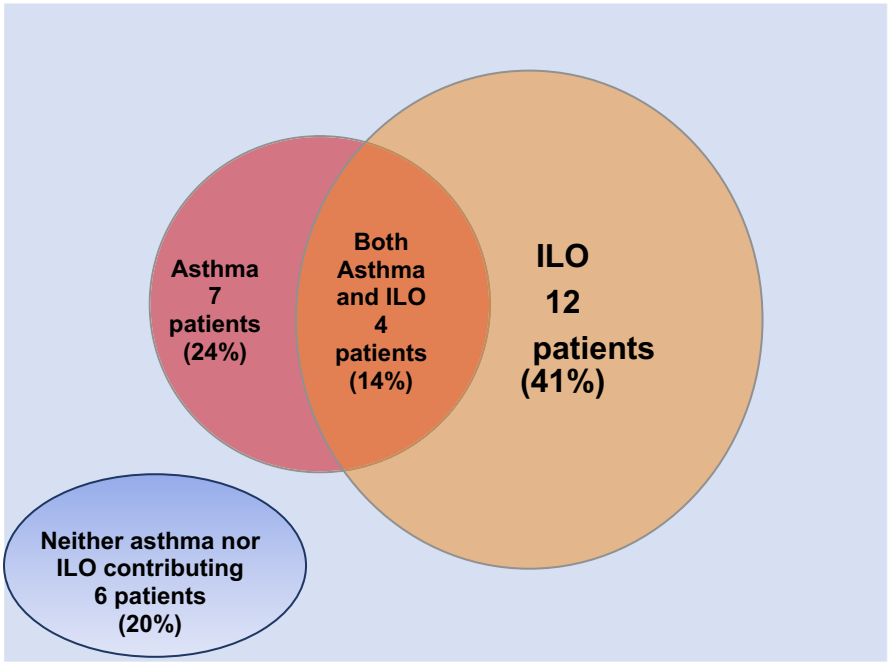

FIGURE 1 Contribution of Asthma and ILO to symptoms in patients with both conditions. ILO, inducible laryngeal obstruction

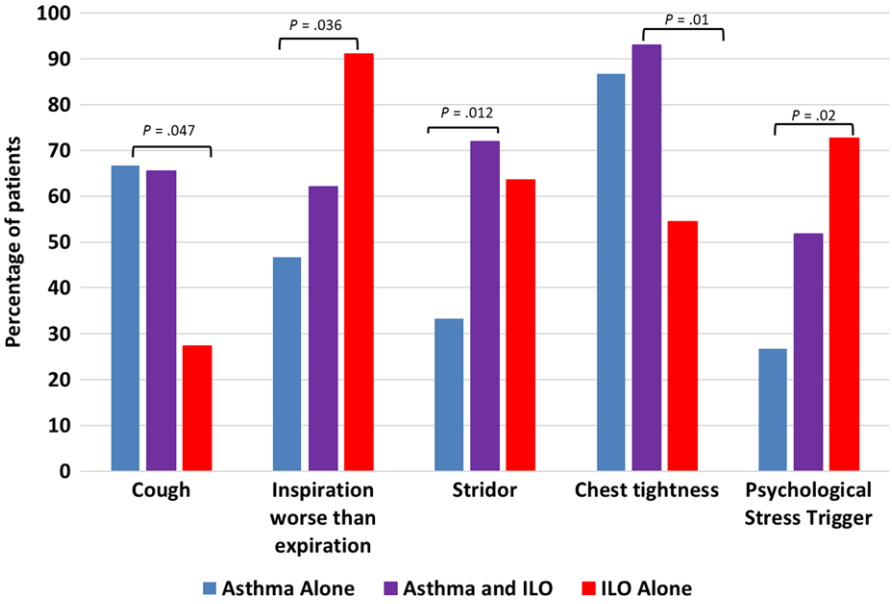

FIGURE 2 Clinical Features in patients with ILO, Asthma and both ILO and Asthma. ILO, inducible laryngeal obstruction

When patients with both ILO and asthma (A+I+) were compared to patients with asthma alone (A+I−), patients with asthma alone had a lower FEV_1 (mean 81% vs 67%, $P = .05$), (Figure 4), while those with both ILO and asthma reported stridor more frequently ($P = .012$) (Figure 2).

When patients with asthma alone were compared to patients with ILO alone, FEV_1 was significantly lower in those with asthma (mean 94.3% vs 66.9%, $P = .003$). Similarly, the forced expiratory ratio was significantly lower in patients with asthma alone (mean 83% vs 64%, $P < .001$). Cough was found to be a more prominent feature in patients with asthma rather than ILO ($P = .047$). Difficulty with inspiration rather than expiration was more commonly seen in the ILO only group ($P = .036$). The presence of psychological stress as a trigger for symptoms was also more commonly seen in the ILO only patient group ($P = .02$). There were no significant differences in frequency of throat symptoms, voice change or vocally traumatic behaviours. (See Table 3 and Figure 2).

Laryngeal dysfunction questionnaire results were not discriminatory in our sample of patients, with abnormal scores seen in patients who had asthma and ILO, as well as patients who had asthma alone (Table S1).

3.4 | Comorbidities accompanying ILO and asthma

In addition to asthma, rhinitis (85%) and gastroesophageal reflux disease (75%) were common comorbidities in the overall cohort. On

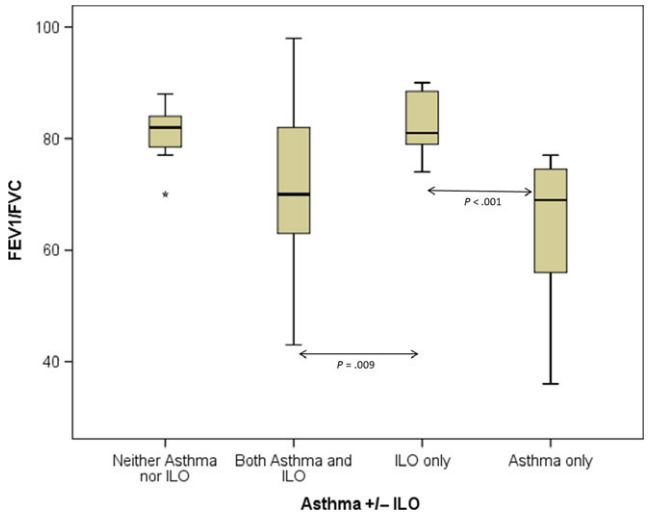

FIGURE 3 Forced Expiratory Ratio (FEV_1/FVC) in patients with asthma, ILO and comorbid asthma and ILO. Hinge: median value, boxes: interquartile range, whiskers: minimum and maximum value. FEV_1, Forced expiratory volume in one-second; FVC, forced expiratory volume; ILO, inducible laryngeal obstruction

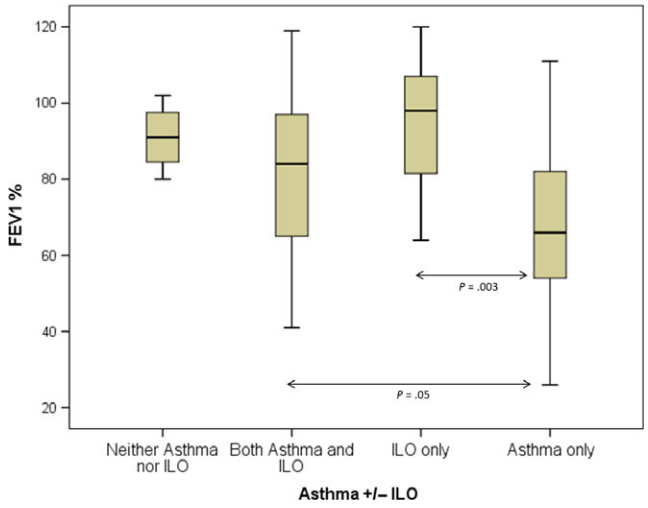

FIGURE 4 FEV_1 in patients with Asthma, ILO and comorbid Asthma and ILO. Hinge: median value, boxes: interquartile range, whiskers: minimum and maximum value. FEV_1, Forced expiratory volume in one-second; ILO, inducible laryngeal obstruction

TABLE 3 Clinical features

	Asthma and ILO (A+I+) n = 29	Asthma alone (A+I−) n = 15	ILO alone (A−I+) n = 11
Respiratory symptoms, n (%)			
Cough*	19 (65.5%)	10 (66.7%)	3 (27.3%)
Unable to breathe beyond a point in the throat	21 (72.4%)	8 (53.3%)	10 (91%)
Inspiration worse than expiration*	18 (62%)	7 (46.7%)	10 (91%)
Choking	18 (62%)	6 (40%)	7 (63.6%)
Stridor*	21 (72%)	5 (33.3%)	7 (63.6%)
Wheeze	23 (79%)	11 (73.3%)	5 (45.4%)
Chest tightness*	27 (93%)	13 (86.7%)	6 (54.5%)
Numbness/dizziness	13 (44.8%)	8 (53.3%)	7 (63.6%)
Rapid onset of symptoms	24 (82.8%)	12 (80%)	10 (91%)
Rapid resolution of symptoms	12 (41.4%)	8 (53.3%)	6 (54.5%)
Relieved by bronchodilators	15 (51.7%)	11 (73.3%)	4 (36.4%)
Emergency department presentation with symptoms, n (%)	19 (65.5%)	11 (73.3%)	6 (54.5%)
Pre-bronchodilator FEV_1%, mean (SD)*	81% (21)	67% (17.1)	94% (17.1)
Pre-bronchodilator FVC %, mean (SD)	88% (19)	81% (21.9)	93% (21)
Forced Expiratory Ratio, mean (SD)**	72% (12.8)	64% (12.3)	83% (6)
Triggers for symptoms			
Odours	17 (56.8%)	6 (40%)	5 (45.5%)
Chemical smell	15 (51.7%)	10 (66.7%)	5 (45.5%)
Smoke	13 (44.8%)	10 (66.7%)	4 (36.4%)
Exercise	18 (62.1%)	8 (53.3%)	5 (45.5%)
Psychological stress*	15 (51.7%)	4 (26.7%)	8 (72.7%)
Workplace trigger	10 (34.5%)	3 (21.4%)	3 (27.3%)

FEV_1, Forced expiratory volume in one-second; FVC, forced expiratory volume.
*$P < .05$, **$P < .001$.

average, each patient had at least 3 comorbid conditions. Validated questionnaires also suggested a high prevalence of dysfunctional breathing. A third of patients described a history of anxiety or depression. However, comorbidities were not significantly different between patients with or without asthma and/or ILO (Table S2).

4 | DISCUSSION

In our patients suspected to have asthma, inducible laryngeal obstruction, or both, almost half had objective evidence of both conditions. Achieving accurate diagnosis was extremely challenging. While a handful of clinical features were statistically more common in one or other condition, none was sufficiently distinctive to guide diagnosis. Furthermore, the frequency of comorbidities and abnormal laryngeal questionnaires could not reliably distinguish patients with each condition. This highlights the necessity of objective testing for both conditions in this clinical scenario. The diagnostic difficulty in our cohort is demonstrated by the mean delay of 5.5 years before achieving an ILO diagnosis.

Although challenging, accurate classification of patients is vitally important. In our 29 patients with both asthma and ILO, 40% were thought to have their symptoms directly attributable to ILO, that is

with stable asthma. As a direct result of our evaluation process, 16 patients had a change to prescribed asthma treatment, either commencing inhaled corticosteroids for uncontrolled asthma or weaning and ceasing inappropriate treatment, often with significant improvements to laryngeal symptoms. These outcomes illustrate the importance of clarifying these two diagnoses.

Our data suggest that many patients with ILO do not fit the previously reported typical profile. Firstly, previous investigators[27,28] have suggested the typical patient with ILO is a female between the second and fourth decades of life with or without a psychological disorder.[29] While our patients were predominantly female, we found a much wider age range. It is true that psychological stress was identified as a trigger for symptoms more commonly in the patients with ILO, but anxiety, depression and dysfunctional breathing were seen with similar prevalence across all our patient groups. Secondly, ILO has been reported to have a higher prevalence among healthcare workers and other occupations where exposure to a variety of irritant chemicals, dusts, mists and fumes may increase risk.[28,30-32] Although some of our patients had identifiable occupational risk factors, the majority of patients did not. We therefore believe that clinicians should be alert to the possibility of ILO even in patients who do not fit the "typical" demographic and clinical profile.

Clinical symptoms of ILO and asthma overlap significantly, and although some clinical features may be more associated with ILO, none have been shown to be specific.[27,33] In our sample, a few key clinical features occurred with a different frequency between patient groups. Stridor and difficulty with inspiration (as compared to expiration) were more prominent in the patients with ILO. Both of these symptoms emphasize the "misbehaving" larynx[5] as the focus of the underlying pathophysiology. Morris and colleagues also previously identified dyspnoea, wheeze, stridor, cough, throat and chest tightness and change in voice as key symptoms. Nevertheless, no single clinical feature in our study was sufficiently accurate to discriminate between ILO and asthma.

To our knowledge, we are the first to report the use a battery of questionnaires, designed to identify and evaluate laryngeal dysfunction as well as to identify relevant comorbidities. However, questionnaire results for laryngeal dysfunction were similarly abnormal in patients (with and without ILO), probably because all patients evaluated in this cohort had already been preselected to have a high clinical probability for ILO. Furthermore, the questionnaires employed had a limited applicability to our particular clinical question. Although the Pittsburgh questionnaire has a high specificity for the diagnosis of ILO, patients with concomitant ILO and asthma were deliberately excluded during its development.[13] The VCD-Q was designed to be a symptom monitoring rather than a diagnostic tool. The dyspnoea index and Newcastle laryngeal hypersensitivity questionnaire were not developed specifically for ILO. Future longitudinal research comparing questionnaire results before and after interventions such as speech pathology, use of neuromodulator agents and treatment of comorbidities may help to validate the utility of these questionnaires.

Specific comorbidities such as chronic rhinitis and gastroesophageal reflux were highly prevalent in our cohort. While they did not distinguish between asthma and ILO, they may serve to trigger both conditions and if detected, should be treated aggressively.[9,27,34,35]

Most authors suggest that formal diagnosis of ILO should be supported where possible by direct visualization of paradoxical vocal fold movement[3,6] as well as the exclusion of alternative diagnoses. Our data show the unreliability of clinical evaluation and support the need for objective testing with direct visualization of ILO in all patients. Fibreoptic laryngoscopy has been criticized as being operator dependent and subjective[36] with a reduced sensitivity if the patient is not experiencing symptoms at the time of examination. Nevertheless at this time, laryngoscopy remains the gold standard for diagnosis. Other methods such as impulse oscillometry[37] and 320 slice CT[38] have considerable drawbacks, due to their limited availability and radiation exposure required, respectively.

Numerous provocation techniques have been used to elicit ILO for diagnosis. Agents have included methacholine, exercise, cold air and irritant challenges, although the sensitivity of these challenges are <50%.[39] Over half of our patients reported exercise as a trigger for their symptoms, but we were unable to undertake exercise provocation at our centre.

We used mannitol, a dry powder inhalant, and have previously reported the ability of mannitol to induce ILO.[19] Direct provocation challenge with histamine or methacholine has traditionally been considered to be more sensitive but less specific for asthma diagnosis.[40,41] We hypothesize that mannitol may be more effective as a laryngeal provoking agent due to its direct irritant effects on the upper airway, although we acknowledge its ability to induce laryngoscopically visualized ILO has not been compared to direct challenge agents. Other measurements obtained during bronchial provocation challenge testing, such as the mean decrease in forced inspiratory flow (%FIF_{50}) has not been found to be a reliable method of detecting (exercise induced) ILO.[42] The inclusion of mannitol provocation testing combined with laryngoscopy in our protocol doubled the detection rate for ILO. We therefore believe provocation testing to form an essential tool in the diagnostic work-up of suspected ILO.

The question as to whether ILO is a physiological consequence of severe asthma or a distinct clinical entity unto itself remains unresolved.[33,36] Uniting mechanisms of airway inflammation and hyperresponsiveness have been suggested[8] as well as an observation of expiratory glottic closure during bronchoconstriction. This may be a compensatory mechanism to increase intrinsic positive end-expiratory pressure and improving alveolar gas exchange.[43,44] However, in our sample, patients with ILO tended to have better lung function, less severe airflow obstruction; and both expiratory and inspiratory ILO were observed, suggesting that ILO was not solely due to airflow limitation or severe asthma.

In normal respiration, the glottic aperture should remain mostly open. Closure of the glottis is mediated by a neuronal reflex arc which may be triggered by proprioceptive, chemical or thermal stimuli.[6] We elected to use the definition of more than 50% adduction

of the vocal folds during expiration in addition to any adduction during inspiration as an indicator of paradoxical movement as has been previously described.[27] We suggest this represents a hypersensitive response, especially as it only occurred in a subset of our cohort. However, we acknowledge there is currently no validated measurement guideline to differentiate normal from abnormal laryngeal responses and the area requires further research.[2]

Two patients in our series demonstrated supraglottic closure, which has been usually described in association with exercise provocation. As we did not undertake exercise provocation, supraglottic laryngeal obstruction may have been underdiagnosed in our cohort.

We recognize several limitations in our study. There was no control group to our observational series. Included patients were highly selected and had a high pre-test probability of some form of laryngeal dysfunction (including inducible laryngeal obstruction), limiting the generalizability of our findings. Detection of paradoxical movement of the vocal folds was by observation which may lead to inter-rater observer variability; although our previous work has demonstrated significant inter-rater agreement.[19] The limited statistical power due to our small sample size may have led to some non-significant results.

5 | CONCLUSION

Asthma and ILO commonly co-exist. We describe a systematic, multidisciplinary assessment process for the diagnosis of asthma and ILO when both conditions are suspected. In patients with both diagnoses, ILO appears to be more clinically symptomatic. In this selected series, laryngeal dysfunction questionnaires were non-discriminatory and a high comorbidity burden was seen in all patients; objective diagnostic testing is therefore essential. Further longitudinal studies evaluating patient outcomes following such diagnosis and assessment processes are warranted.

ACKNOWLEDGEMENTS

The authors thank the Alfred lung function laboratory for invaluable assistance during mannitol challenge testing, Ms Fiona Hore-Lacy for her assistance in the set-up of the RedCAP platform and Catherine Martin for statistical advice.

CONFLICTS OF INTEREST

Joy Lee has delivered educational talks for GlaxoSmithKline and Astra Zeneca. Michael Abramson holds investigator initiated grants from Pfizer and Boehringer-Ingelheim for unrelated research. He has also received assistance with conference attendance from Sanofi. Mark Hew has undertaken contracted research for AstraZeneca, Sanofi, Novartis, & GlaxoSmithKline; delivered Educational talks for GlaxoSmithKline, AstraZeneca & Novartis; Participated on advisory boards/consultancies for AstraZeneca, GSK & Seqirus; for all of which his employer (Alfred Health) has been reimbursed.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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SUPPLEMENTARY MATERIAL

Table 1. Laryngeal Dysfunction Questionnaire Results

	Asthma and ILO (A+I+) N=29	**Asthma alone (A+I-) N=15**	**ILO alone (A-I+) N=11**	**Reference Values**
Pittsburgh VCD index(110)	5.5±1.7	5.6±2.1	4.8±3.2	> 4 = positive predictive value of 96% for VCD
Dyspnoea Index (112)	24.6±7.1	25.5±8.1	28.4±7.5	>10 Abnormal
VCD-Q (111)	41.1±7.3	42.47±10.4	42.36±7.3	Not tested as a diagnostic questionnaire, but patients with score >40 are more likely to have VCD (ILO)
Newcastle Laryngeal Hypersensitivity Questionnaire (113)	13.7±3.4	13.8±4.2	12.9±3.5	>17.1 Normal

VCD=Vocal cord dysfunction; ILO=inducible laryngeal obstruction; SD = standard deviation
All data presented as (mean ±SD)

Table 2. Comorbidities

	Asthma and ILO (A+I+) N=29	**Asthma alone (A+I-) N=15**	**ILO alone (A-I+) N=11**
Obesity (BMI≥30)	12 (41.4%)	7 (46.7%)	6 (54.5%)
Chronic Rhinitis	25 (56.8%)	14 (63.6%)	8 (72.7%)
Obstructive Sleep Apnoea	5 (17.2%)	2 (13.3%)	2 (18.2%)
Gastroesophageal Reflux Disease	21 (72.4%)	13 (86.7%)	7 (63.6%)
Anxiety (Clinician diagnosed)	14 (48.3%)	7 (46.7%)	3 (27.3%)
Depression (clinician diagnosed)	12 (41.3%)	6 (40%)	4 (36.4%)
Dysfunctional breathing Nijmegen score >23	13 (44.8%)	9 (60%)	6 (54.5%)
Chemical Sensitivity Scale and Sensory Hyper-reactivity (CSS - SHR) >43	9 (31%)	6 (40%)	3 (27.3%)

All data presented as n (proportion)

5.2 Summary of Findings from Chapter Five

In a population of patients with suspected asthma and ILO, 42% were able to have both diagnoses objectively demonstrated. Asthma alone was present in 22% and ILO alone in 16%. ILO was diagnosed on laryngoscopy either at baseline or following mannitol provocation. When asthma was present, chest tightness was a more common presenting symptom. Stridor, difficulty with inspiration and symptoms triggered by psychological stress were more frequently seen among patients with ILO. Patients with asthma had more airflow obstruction on spirometric testing. Comorbidities such as allergic rhinitis and gastroesophageal reflux were commonly seen among all patients. Questionnaires did not reliably differentiate the two diagnoses. This study emphasises the importance of systematic and objective diagnostic procedures to reliably identify and discriminate between asthma and ILO.

Chapter Six: Predictors of VCD within a Difficult Asthma Population

6.1 Introduction

This study examined a consecutive series of patients with difficult-to-treat asthma undergoing multidisciplinary systematic assessment at the Alfred hospital. Patients with suspected vocal cord dysfunction underwent laryngoscopy for diagnostic confirmation. Relationships between VCD and clinical factors (demographics, asthma parameters and other comorbidities) were identified using multiple logistic regression.

Lee J, Denton E, Hoy R, Tay TR, Bondarenko J, Hore-Lacy F, Radhakrishna N, O'Hehir RE, Dabscheck El, Abramson M and M Hew. Paradoxical vocal fold motion in difficult asthma is associated with dysfunctional breathing and preserved lung function. Journal of Allergy and Clinical Immunology in Practice (INPRACTICE-D-19-01010). 2020;8(7):2256-2262. July 2020.

Original Article

Paradoxical Vocal Fold Motion in Difficult Asthma Is Associated with Dysfunctional Breathing and Preserved Lung Function

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What is already known about this topic? Patients with "difficult-to-treat" asthma may also have comorbidities, such as vocal cord dysfunction, which are associated with worse asthma outcomes, such as exacerbation risk, high symptom burden, and poorer quality of life.

What does this article add to our knowledge? Clinical features associated with vocal cord dysfunction amongst patients with difficult asthma include dysfunctional breathing and preserved lung function.

How does this study impact current management guidelines? Identifying and treating dysfunctional breathing alongside vocal cord dysfunction should be included in management guidelines for patients with difficult asthma and is likely to be essential to improve patient outcomes.

BACKGROUND: Many patients with difficult asthma also have coexisting vocal cord dysfunction (VCD), evident by paradoxical vocal fold motion (PVFM) on laryngoscopy.
OBJECTIVE: Among patients with difficult asthma, we sought to identify clinical features associated with laryngoscopy-diagnosed PVFM.
METHODS: Consecutive patients with "difficult asthma" referred by respiratory specialists underwent systematic assessment in this observational study. Those with a high clinical suspicion for VCD were referred for laryngoscopy, either at rest or after mannitol provocation. Statistical analyses were performed to identify clinical factors associated with PVFM, and a multivariate logistic regression model was fitted to control for confounders.
RESULTS: Of 169 patients with difficult asthma, 63 (37.3%) had a high clinical probability of VCD. Of 42 who underwent laryngoscopy, 32 had PVFM confirmed. Patients with PVFM more likely had preserved lung function (prebronchodilator forced expiratory ratio 74% ± 11 vs 62% ± 16, $P < .001$); physiotherapist-confirmed dysfunctional breathing (odds ratio [OR] = 5.52, 95% confidence interval [CI]: 2.4-12.7, $P < .001$), gastro-oesophageal reflux (OR = 2.6, 95% CI: 1.16-5.8, $P = .02$), and a lower peripheral eosinophil count (0.09 vs 0.23, $P = .004$). On multivariate logistic regression, independent predictors for PVFM were dysfunctional breathing (OR = 4.93, 95% CI: 2-12, $P < .001$) and preserved lung function (OR = 1.07, 95% CI: 1.028-1.106, $P < .001$).
CONCLUSION: Among specialist-referred patients with difficult asthma, VCD pathogenesis may overlap with dysfunctional breathing but is not associated with severe airflow obstruction. Dysfunctional breathing and preserved lung function may serve

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Abbreviations used
ACT- Asthma Control Test
AQLQ- Asthma-related quality of life
CI- Confidence interval
ENT- Ear, nose, and throat
FER- Forced expiratory ratio
FEV1- Forced expiratory volume in 1 second
HADs- Hospital Anxiety and Depression Scale
OR- Odds ratio
PVFM- Paradoxical vocal fold motion
VAO- Variable airflow obstruction
VCD- Vocal cord dysfunction

as clinical clues for the presence of VCD. © 2020 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2020;■:■-■)

Key words: *Asthma; Larynx; Paradoxical vocal fold motion; Vocal cord dysfunction*

"Difficult asthma" is a diagnosis applied to patients who despite high-dose preventer treatment continue to experience a high symptom burden or frequent exacerbations.[1] Misdiagnosis, coexisting comorbidities, nonadherence to medication, and biologically severe disease may all contribute to difficult asthma. Systematic multidisciplinary assessment is therefore beneficial to identify these contributory factors, distinguish biologically severe asthma from difficult asthma, and improve patient outcomes.[2-5]

Vocal cord dysfunction (VCD), also known as the glottic form of inducible laryngeal obstruction, is an important comorbidity in difficult asthma. Symptoms of dyspnoea, throat tightness, dysphonia, and stridor arise from intermittent airway obstruction caused by paradoxical vocal fold motion (PVFM), which can be visualized on laryngoscopy. This paradoxical adduction is thought to be due in part to laryngeal hypersensitivity,[6] which may be triggered by irritants such as odors and strong emotions as well as risk factors such as laryngopharyngeal reflux or post-nasal drip.[7] Patients with concomitant VCD and asthma have worse asthma outcomes including increased symptoms, poorer quality of life, and more exacerbations.[8]

VCD may affect as many as 30% to 50% of patients with difficult asthma in some series,[9] serving either as a coexisting or alternative diagnosis. VCD is frequently under-recognized, even by respiratory specialists,[10] leading to delays in diagnosis and inappropriate treatment.[10-12] We have previously reported on the challenges in distinguishing VCD from asthma, even with classic signs and symptoms such as stridor and inspiratory difficulty, which highlights the need to visualize PVFM on laryngoscopy to confirm VCD diagnosis.[13]

This study aimed to identify clinical features of laryngoscopy-diagnosed VCD, among patients with difficult asthma referred by respiratory specialists for systematic multidisciplinary assessment.

METHODS

Our tertiary hospital in Melbourne, Australia, undertakes systematic assessment of adults with difficult asthma referred by respiratory and allergy specialists. Specialists may refer patients with asthma that is difficult to control due to any of the following reasons: diagnostic dilemma; poor symptom control; frequent or serious exacerbations; poor lung function; or patient factors, such as poor adherence or psychosocial concerns.[14]

TABLE I. Comorbidity questionnaires

Comorbidity	Questionnaire
Chronic rhinosinusitis	Sinonasal Questionnaire (SNQ)[19]
	Sinonasal Outcome Test (SNOT-22)[20]
Allergic rhinitis	Rhinitis Control Assessment Test (RCAT)[21]
Gastroesophageal reflux disease	Gastroesophageal Reflux Questionnaire (GERD-Q)[22]
Obstructive sleep apnoea	Berlin Questionnaire[23]
Depression	Hospital Anxiety and Depression Scale (HADs)[24]
Anxiety	HADs[24]
Dysfunctional breathing	Nijmegen score[25]
Vocal cord dysfunction	Pittsburgh VCD Index[26]
	Vocal Cord Dysfunction Questionnaire (VCD-Q)[27]

Patients are assessed over 3 visits conducted over a 6-month period, as previously described. Consecutive patients who completed assessment between June 1, 2014, and March 9, 2018, were eligible for inclusion in this analysis. This research was approved by the Alfred Health Ethics committee (Reference number 285/15).

Systematic assessment

A standardized electronic clinic template on REDCap (Research Electronic Data Capture), a secure web-based application,[15] was used to collect baseline demographics and clinical data as previously described.[16] The template included a comprehensive asthma history and questionnaires to assess asthma control (Asthma Control Test or ACT),[17] asthma-related quality of life (AQLQ),[18] and 8 comorbidities,[16] as listed in Table I. Permission from questionnaire authors was obtained.

The diagnosis of asthma was confirmed by stepwise testing for the presence of variable airflow obstruction (VAO) using bronchodilator reversibility on spirometry, peak flow variability, or (when safe and appropriate) positive bronchial provocation challenge test with mannitol (Aridol; Pharmaxis, Sydney, Australia).

Further investigations for phenotyping included assessment of atopic status by skin prick testing or *in vitro* allergen specific IgE, fractional exhaled nitric oxide measurement, total serum IgE, and peripheral blood eosinophil count. All patients underwent asthma nurse assessment of inhaler technique, and were given a written asthma action plan and an electronic monitoring device for objective adherence monitoring if a device was compatible with their inhalers.[28]

Patients were classified as having a high clinical probability of VCD if compatible clinical symptoms were present, the specialist clinician had a high index of suspicion after systematic assessment, and supported by an abnormal Pittsburgh VCD index. As a gold standard of VCD diagnosis, both compatible clinical history and objective paradoxical vocal cord adduction seen on laryngoscopy were required. Laryngoscopy was performed either by an ear, nose, and throat (ENT) specialist or through the specialized middle airway clinic protocol, which also included nasoendoscopy with laryngeal provocation using mannitol when required.[13,29] Mannitol provocation was used if vocal cord examination was normal at rest. Paroxysmal movement was defined as abnormal adduction of the vocal cords on inspiration or greater than 50% and sustained adduction of vocal cords observed in expiration.

Patients were referred for further investigation of dysfunctional breathing if compatible clinical symptoms were present and the assessing clinician had a high index of suspicion. Dysfunctional breathing was diagnosed by specialized single physiotherapist assessment comprising assessment for mouth breathing, hyperventilation (resting elevated respiratory rate), thoracic dominance, thoracoabdominal asynchrony, and accessory muscle use.[30]

The diagnoses of other comorbidities were made by criteria outlined by Tay et al[3] and summarized as follows. Chronic rhinosinusitis was diagnosed by the presence of clinical symptoms and visible sinus opacification on computed tomography, or ENT examination. Allergic rhinitis was diagnosed by clinical assessment by an allergist and positive skin prick test or serum specific IgE to common aeroallergens. Obstructive sleep apnoea was indicated by the presence of clinical symptoms and Berlin questionnaire score greater than or equal to 2, or a previous positive polysomnogram. Gastroesophageal reflux disease was determined by the presence of reflux symptoms and gastroesophageal reflux questionnaire score greater than 2, or a known history of reflux disease on acid suppression treatment. Anxiety and depression were diagnosed based on clinical symptoms and Hospital Anxiety and Depression Scale (HADs) score (either anxiety, depression, or both) greater than or equal to 11, or a known history of anxiety or depression currently on medication.

Statistical analysis

Data analysis was performed using SPSS version 22 (IBM, Armonk, NY). Descriptive analyses included categorical variables that are presented as percentages and frequency, and continuous variables that are presented as mean or median values with standard deviation or interquartile range, respectively. Univariate analyses identified clinical factors associated with VCD. Unpaired *t*-tests or Wilcoxon rank sum tests were used to compare continuous variables, and χ^2 or Fisher's exact tests to compare categorical variables, depending on data distribution. Logistic regression was used to identify relationships between suspected clinical factors (from a previous study[31]) and VCD. Results are presented as odds ratios (ORs) with 95% confidence intervals (CIs), with a *P* value of less than .05 reported as significant. These factors were then adjusted for possible confounders, including age, gender, and forced expiratory ratio (FER), in a multivariate model, with results presented as adjusted ORs. Sensitivity analysis including patients with missing data was performed and included in the results.

RESULTS

Between May 1, 2015, and March 9, 2018, 169 consecutive patients were assessed for difficult asthma. Of these, 63 (37.3%) had a high clinical probability of VCD based on clinical assessment supported by questionnaire results. Of these, 17 patients did not proceed to laryngoscopy (Figure 1): 5 declined laryngoscopy, 7 failed to attend laryngoscopy, 1 was assessed by an ENT surgeon external to our hospital, and the remaining 4 were not thought to clinically have VCD on subsequent review, with symptom improvement after treatment of chronic rhinosinusitis, gastroesophageal reflux disease, obesity with bariatric surgery, or severe asthma with monoclonal antibody therapy.

The other 46 underwent laryngoscopy either at rest or after laryngeal provocation with mannitol,[29] and confirmed PVFM was observed in 32. Only these patients with confirmed paradoxical movement on laryngoscopy were included for primary statistical analysis. Among 14 patients with observable *normal* vocal fold movement despite provocation, 6 had alternative laryngeal pathology identified on laryngoscopy, including 1 with vocal fold paresis, 2 with muscle tension dysphonia, 1 with fungal laryngitis, and 2 with laryngeal pachydermia and laryngopharyngeal reflux. These patients were treated appropriately with speech, antifungal, and reflux therapy, respectively.

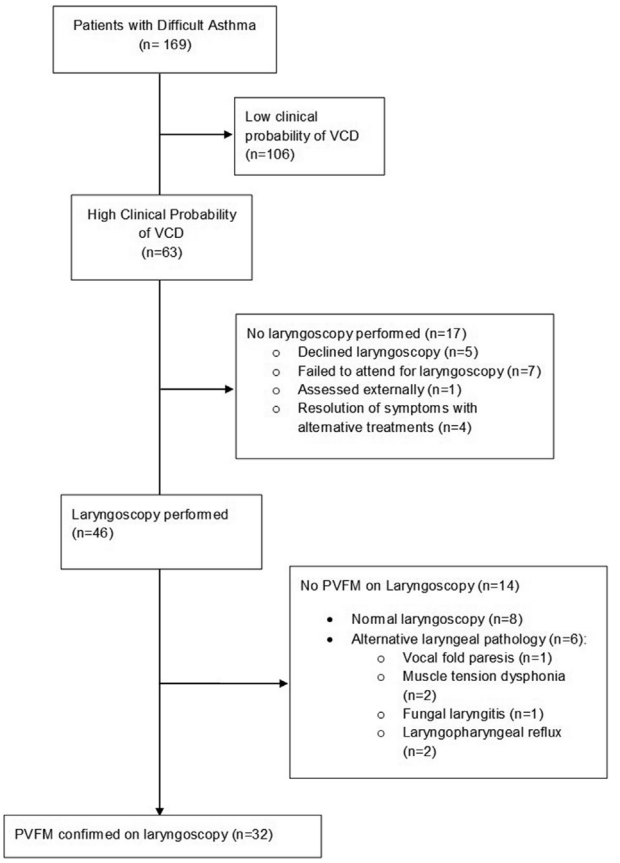

FIGURE 1. CONSORT diagram depicting flow of patients. *PVFM*, Paradoxical vocal fold motion; *VCD*, vocal cord dysfunction.

Baseline demographics of the patients are described in Table II. Clinical asthma characteristics and asthma comorbidities are described in Tables III and IV, respectively.

On univariate analysis, baseline demographics including age, gender, body mass index, and smoking status did not differentiate between patients with or without PVFM. VAO was demonstrated objectively in the majority (86%) of patients. The proportion of patients with VAO was not different among patients with and without demonstrable PVFM. Of the 23 patients without demonstrable VAO, 9 patients with negative bronchial provocation challenge tests had VCD as an alternative diagnosis to severe asthma and had their inhaled corticosteroid dose reduced or ceased. Three patients had dysfunctional breathing as an alternative diagnosis and also had asthma medication reduced. Five patients had severe fixed airflow obstruction consistent with chronic obstructive pulmonary disease and were not eligible for bronchial provocation challenge testing. One patient's symptoms were attributed to obesity with improvement in symptoms after bariatric surgery. The remaining 5 patients failed to return peak flow charts or declined bronchial provocation testing.

TABLE II. Baseline characteristics

Baseline characteristics	Total	Patients with PVFM on laryngoscopy (n = 32)	Patients without PVFM (n = 137)	*P* value
Age (y), mean (SD)	52 (14), range 19-80	50 (12.7)	52 (14.6)	.491
Gender, n (%)				
Female	112 (66%)	24 (75%)	88 (64%)	.246
BMI (kg/m^2), mean (SD)	32 (8.4)	33 (6.7)	31 (8.7)	.55
BMI ≥30, n (%)	91 (54%)	20 (63%)	71 (52%)	.275
Smoking status, n (%)				.830
Never	95 (57%)	16 (52%)	79 (585)	
Ex-smoker	65 (39%)	14 (45%)	51 (37%)	
Current smoker	7 (4%)	1 (3.2%)	6 (4%)	
Unemployed	81 (49%)	14 (46.7%)	67 (49%)	.797

BMI, Body mass index; *PVFM*, paradoxical vocal fold motion; *SD*, standard deviation.

TABLE III. Asthma characteristics

	Total	Patients with PVFM on laryngoscopy (n = 32)	Patients without PVFM (n = 137)	*P* value
Asthma questionnaire results at visit 1				
ACT, mean (SD)	13.9 (5)	12.39 (5)	14.25 (5)	.064
AQLQ, mean (SD)	4.2 (1.24)	3.9 (1.24)	4.27 (1.24)	.136
Age at asthma diagnosis, mean (SD)	22 (20)	20 (13)	22 (20)	.64
Asthma diagnosis				
Variable airflow obstruction demonstrated, n (%)	146 (86)	28 (88)	118 (86)	.839
Peak flow variability, n (%)	30 (17.8)	7 (22)	23 (17)	.5
Bronchodilator reversibility, n (%)	98 (58)	16 (50)	83 (60)	.309
Bronchial provocation challenge, n (%)	17 (10.1)	5 (16)	12 (9)	.245
Lung function characteristics				
Prebronchodilator FEV_1 (%predicted), mean (SD)	67 ± 23	79 (18)	65 (23)	**<.001**
Prebronchodilator FVC (%predicted), mean (SD)	81 ± 17	85 (16)	80 (17)	.130
Prebronchodilator FER, mean (SD)	64.5 ± 15.5	74 (11)	62 (16)	**<.001**
FER <70% (airflow obstruction), n (%)	96 (57)	13 (41)	83 (61)	**.040**
Severe airflow obstruction (FEV_1 <40% predicted)	19 (11)	0 (0)	19 (14)	**.026**
Asthma phenotype				
Atopic, n (%)	95 (58)	17 (57)	78 (58)	.911
Blood eosinophils (×10^9/L), median (IQR)	0.19 (0.37)	0.09 (0.23)	0.23 (0.41)	**.004**
IgE (kU_A/L), median (IQR)	124.5 (422)	70 (371)	134 (475)	.119
FeNO (ppb), median (IQR)	24 (32)	23 (19)	24 (34)	.708
Asthma severity				
GINA treatment step 4 or 5,* n (%)	146 (86)	27 (84)	119 (86)	.772
Exacerbations, mean (SD)				
Burst of oral corticosteroids	2.5 (2)	2.9 (1.7)	2.4 (2)	.257
Unscheduled GP visit	2.3 (3.5)	2.9 (3)	2 (4)	.265
ED presentation	0.7 (1.4)	0.9 (1)	0.7 (1.4)	.329
Hospitalization	0.37 (0.9)	0.34 (0.5)	0.3 (0.5)	.513
Adherence to prescribed preventer				
Self-reported adherent >75%, n (%)	144 (85)	29 (94)	115 (84)	.190
Percentage adherent as measured by EMD (median, IQR)	75% (27)	53% (56)	77% (21)	**.043**

ACT, Asthma Control Test;[17] *AQLQ*, asthma-related quality of life; *ED*, emergency department; *EMD*, electronic monitoring device; *FeNO*, fractional exhaled nitric oxide; *FER*, forced expiratory ratio; *FEV_1*, forced expiratory volume in 1 second; *FVC*, forced vital capacity; GINA, Global Initiative for Asthma; *GP*, general practitioner; *ICS*, inhaled corticosteroid; *IQR*, interquartile range; *PVFM*, paradoxical vocal fold motion; *SD*, standard deviation.
P values in bold indicate results significant to level of $P < .05$.
*GINA step 4: moderate-to-high dose ICS; GINA step 5: add on therapy including oral corticosteroids.

A higher forced expiratory volume in 1 second (FEV_1) was seen among patients with PVFM compared with those without (79% compared with 65% predicted, $P < .001$), with less airflow obstruction (mean FER 74% vs 62%, $P < .001$). Peripheral blood eosinophil count was lower among patients with PVFM (median 0.09 compared with 0.23×10^9/L, $P = .004$). Despite these differences, exacerbation frequency was similar among patients with and without PVFM. Dysfunctional breathing (50% vs 15%, $P < .001$) and gastroesophageal reflux disease (66% vs 42%, $P = .017$) were key comorbidities more frequently present

TABLE IV. Comorbidities

	Total	Patients with PVFM on laryngoscopy (n = 32)	Patients without PVFM (n = 137)	*P* value
Allergic rhinitis	90 (53%)	20 (63%)	70 (51%)	.244
Chronic rhinosinusitis	65 (39%)	12 (38%)	53 (39%)	.901
Dysfunctional breathing	37 (22%)	16 (50%)	21 (15%)	**<.001**
Obstructive sleep apnoea	52 (31%)	11 (34%)	41 (30%)	.624
Gastroesophageal reflux disease	79 (47%)	21 (66%)	58 (42%)	**.017**
Chronic obstructive pulmonary disease	18 (11%)	1 (3%)	17 (13%)	.200
Anxiety	43 (26%)	11 (34%)	32 (24%)	.242
Depression	50 (30%)	13 (41%)	37 (28%)	.157
Comorbidity questionnaire results				
SNQ[19]	1.44 (0.9)	1.4 (0.8)	1.5 (1)	.609
SNOT-22[20]	39 (21)	39 (22)	39 (22)	.954
RCAT[32]	21 (5)	20 (5)	21 (5)	.424
Nijmegen score[25]	23 (12)	25 (13)	22 (14)	.168
VCD-Q[27]	40 (9)	44 (8)	38 (9)	**.008**
Pittsburgh VCD index[26]	2.6 (2.4)	4 (2)	2.3 (2.3)	**<.001**
HADs-A[24]	8 (5)	9 (6)	7 (5)	.091
HADs-D[24]	6 (4)	7.6 (5)	5 (4)	**.006**

All questionnaire results reported as mean (SD) unless otherwise stated.
P values in bold indicate results significant to level of $P < .05$.
HADs-A, Hospital Anxiety Scale; *HADs-D*, Hospital Depression Scale; *PVFM*, paradoxical vocal fold motion; *RCAT*, Rhinitis Control Assessment Test; *SD*, standard deviation; *SNOT-22*, Sinonasal Outcome Test; *SNQ*, Sinonasal Questionnaire; *VCD-Q*, Vocal Cord Dysfunction Questionnaire.

TABLE V. Clinical predictors for VCD

Clinical factor	Odds ratio (95% CI)	Adjusted odds ratio* (95% CI)	*P* (for adjusted OR)
Dysfunctional breathing	5.524 (2.4-12.7)	4.93 (2.0-11.96)	<.001
Depression	1.78 (0.8-4)	1.7 (0.724-3.998)	.222
Anxiety	1.64 (0.7-3.76)	1.38 (0.58-3.32)	.47
Gastroesophageal reflux disease	2.6 (1.16-5.8)	2.089 (0.871-5)	.099
Forced expiratory ratio	1.061 (1.027-1.097)	1.067 (1.028-1.106)	<.001

CI, Confidence interval; *OR*, odds ratio; *VCD*, vocal cord dysfunction.
*Adjusted for baseline demographics (Table I) including age, gender, spirometry parameters.

in the PVFM population. Patients with PVFM also had higher HADs-D scores ($P = .006$) although diagnoses of depression were not more frequent. Patients with PVFM had higher scores in both the Pittsburgh Vocal Cord Index[26] and the Vocal Cord Dysfunction Questionnaire.[27]

Although self-reported adherence was similar among both patient groups (94% vs 84% of patients reported taking their inhalers more than 75% of the time, $P = .19$), when data from objective electronic monitoring devices were analyzed, patients with PVFM had lower rates of adherence to their preventer inhaler (median taking of prescribed doses 53% of the time) compared with those without PVFM (77%, $P = .043$).

Key clinical comorbidities (dysfunctional breathing, anxiety, depression and gastroesophageal reflux disease, lung function) were included in the multivariate logistic regression model, controlling for potential confounders of age and gender. Peripheral eosinophil count was not included in multivariate analysis because of collinearity with markers of airflow obstruction. Dysfunctional breathing was independently associated with VCD (OR = 4.93, 95% CI: 2.0-12.0, $P < .001$), as was preserved lung function: for every unit increase in FER, the odds of identified VCD increased by 3.8% (95% CI: 1.028-1.106, $P < .001$) (Table V).

We conducted a sensitivity analysis to include all 63 patients with a high index of clinical suspicion of VCD. This analysis confirmed relationships between VCD and less severe FEV_1 and FER (72% predicted vs 64% predicted, $P = .025$, and 70% predicted vs 61% predicted, $P < .001$, respectively), dysfunctional breathing ($P = .005$), and gastroesophageal reflux disease ($P = .006$). In addition, there were also associations with female gender (76% vs 69%, $P = .036$), oral corticosteroid use in the prior 6 months (2.94 vs 2.24 courses, $P = .03$), and poorer asthma control (Asthma Control Questionnaire 6: 2.7 vs 2.2, $P = .027$) and quality of life scores (AQLQ: 3.87 vs 4.4, $P = .006$). However, there was no significant association with blood eosinophil level ($P = .16$).

DISCUSSION

Key findings

VCD is an important comorbidity among patients with difficult asthma, because it is frequently found in this population and its symptoms closely mimic those of asthma.[13] VCD was found to often coexist with asthma, as demonstrated by the presence of objective VAO in most patients. In this sample, we examined the association between clinical factors and VCD. VCD was defined by laryngoscopic-visualized PVFM. Dysfunctional breathing was associated with VCD, as was preserved lung function. These findings have implications for the pathogenesis of VCD in difficult

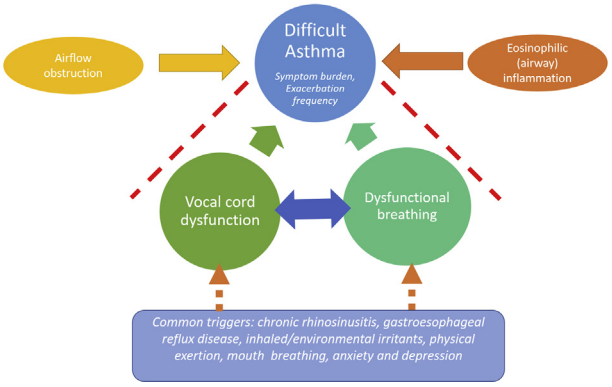

FIGURE 2. Proposed relationships between vocal cord dysfunction, dysfunctional breathing, and difficult asthma (as defined by symptom burden and exacerbation frequency). Dashed arrow = suspected clinical associations.[33,37] Dashed red line = lack of association in this study.

asthma and may also help to identify patients in this cohort in whom VCD is most likely to be present.

Implications for VCD pathogenesis

The association of VCD with less severe airflow obstruction in this difficult asthma cohort was intriguing. It has previously been suggested that unstable and severe airflow obstruction in asthma may induce VCD through a variety of putative mechanisms including high inspiratory flow rates, enhanced perception of symptoms, hyperventilation-induced airway dryness, and emotion-triggered cholinergic and sympathetic activity.[33,34] Our findings imply instead that severe lower airway obstruction may not be an essential part of VCD pathophysiology in difficult asthma.

The significant association of VCD with dysfunctional breathing in difficult asthma supports a relationship between the 2 conditions. Recognition of both comorbidities among patients with difficult asthma is increasing.[35] However, many studies have focused separately on either VCD or dysfunctional breathing. Some researchers have suggested that both conditions should be recognized as somatoform respiratory disorders—functional disorders of respiratory regulation.[36] Others have suggested that VCD represents an extrathoracic form of dysfunctional breathing.[37] In the context of our current data in difficult asthma, the relationship between VCD and dysfunctional breathing may well be bidirectional, with both conditions potentially increasing symptom burden and exacerbation frequency (Figure 2). The identification of one comorbidity should thus prompt a search for the other. Also, based on our data, these relationships appear unrelated to lower airway obstruction or eosinophilic airway inflammation (Figure 2). Interventional study designs are needed to explore these relationships further.

In addition, there may be triggers common to both dysfunctional breathing and VCD.[6] Some of these potential triggers were examined in our study, namely chronic rhinosinusitis, gastroesophageal reflux disease, obstructive sleep apnoea, anxiety, and depression, but no significant relationships between these variables and the presence of VCD were found on multivariate analysis.

In this analysis, the Nijmegen score was a poor discriminator for the presence or absence of dysfunctional breathing. Previous work at our center has demonstrated the limitations of the Nijmegen score in identifying dysfunctional breathing, highlighting the importance of a more objective diagnosis of dysfunctional breathing by an experienced physiotherapist, as undertaken in this and other studies.[30,38] A sensitivity and specificity analysis comparing the performance of the Nijmegen score to physiotherapy assessment among this difficult patient population was 21% and 42%, respectively.

Implications for clinical practice

Despite significantly better lung function in patients with VCD, the symptoms and quality of life impairment, frequency of asthma exacerbations, and health care utilization were similar to those without VCD. This is consistent with previous studies indicating the impact of VCD on patient outcomes and health care utilization.[39] Patients with VCD were also more likely to have lower eosinophil counts. These findings together suggest that VCD may be more likely to contribute to symptom burden and exacerbation frequency in patients with preserved lung function and minimal (eosinophilic) airway inflammation. Although self-reported adherence to preventer asthma treatment was similar among patients with and without VCD, when examined objectively, patients with VCD had lower rates of adherence to their prescribed preventer. Although we have previously reported the inaccuracy of self-reported adherence within this population,[28] this discrepancy may reflect the poor response of VCD symptoms to inhaled asthma therapies.

Strengths and limitations of this study

This is the first study to demonstrate a substantial relationship between VCD and dysfunctional breathing in difficult asthma. Our patients with difficult asthma underwent a standardized and systematic assessment, and follow-up of patients was close to 100%. We defined the diagnosis of VCD with objective visualization of PVFM at laryngoscopy. The diagnosis of dysfunctional breathing was established by standardized physiotherapist assessment[30] rather than with the Nijmegen questionnaire, the use of which did not demonstrate a relationship with VCD in a previous analysis.[40] There is also an inherent limitation in defining these clinical entities, particularly dysfunctional breathing, due to a lack of agreed gold standard diagnostic criteria and definitions, which leads to reliance on consensus statements and expert opinions.

We also recognize the limitations inherent to an observational case series—that associations do not necessarily imply causation. In this pragmatic clinical protocol, only patients with a clinical suspicion of VCD or dysfunctional breathing were referred for laryngoscopy and/or physiotherapy assessment. Furthermore, not all patients referred for laryngoscopy underwent the procedure. Patients with more severe airflow obstruction may have been thought to be less likely to have VCD or may have been thought to poorly tolerate further investigation (ie, laryngoscopy and/or provocation). Thus, our results may represent an underestimation of the presence of PVFM in difficult asthma.

Future directions

Although observational studies in difficult asthma have highlighted both VCD and dysfunctional breathing as important comorbidities among this population, further studies are required to determine underlying pathophysiological mechanism(s) and to confirm this inter-relationship in a larger sample and within other difficult asthma groups. Furthermore, randomized controlled trials are needed to determine which patients would benefit most from therapy and which therapies

are the most effective, whether this would include speech therapy, physiotherapist directed breathing retraining, or both. Traditionally, patients with VCD are referred to speech therapists and patients with dysfunctional breathing are referred to physiotherapists, but other treatment paradigms may warrant exploration. Further item analyses of asthma symptom questionnaire scores (eg, ACT and AQLQ) may also help to further identify distinguishing symptoms among patients with VCD compared with asthma.

Conclusion

Among patients with difficult asthma, VCD and dysfunctional breathing are likely to overlap, suggesting that their pathogeneses may be related. Patients with difficult asthma and preserved lung function should be examined carefully for the presence of both dysfunctional breathing and VCD. They should also be considered for interventions such as breathing retraining or speech therapy, in addition to standard asthma management.

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6.2 Summary of Findings from Chapter 6

Among 169 consecutive patients, 32 (19%) had laryngoscopic evidence of VCD. These patients had less airway obstruction and less eosinophilic inflammation, despite similar levels of asthma exacerbations, symptom control and quality of life to the other patients. On multivariate analysis, physiotherapist-diagnosed dysfunctional breathing (adjusted OR 4.93, 95%CI 2 -12, p<0.001) and preserved lung function (adjusted OR 1.067 95%CI (1.028 - 1.106 p<0.001) were independently associated with VCD.

This study supports a relationship between VCD and dysfunctional breathing, a term which describes abnormal breathing patterns encompassing hyperventilation, periodic deep sighing, upper chest predominant breathing, and asynchronous breathing. This relationship raises the possibility of shared pathogenic pathways between the two conditions. The co-existence of both conditions in patients with difficult-to-treat asthma may compound the burden of asthma symptoms. Since patients with VCD had better lung function than those without VCD, it is unlikely that airflow obstruction *per se* induces VCD.

Airway obstruction may also occur upstream and/or downstream from the middle airway, and the presence of one or both of these may increase the risk of VCD being present. In obstructive sleep apnoea, the upper airway is often described as a tube with a collapsible segment, analogous to a Starling-resistor model. Airway collapse and reduced airflow occur when the upstream pressure in the nasal segment and surrounding pressure is greater than downstream pressure in the hypopharynx. Complete airflow obstruction occurs when the surrounding pressure exceeds both these upstream and downstream pressures.(148) Interestingly, in this study, there was no significant difference in presence of obstructive

sleep apnoea between patients with or without VCD, although this may have been affected by the small sample size.

Further downstream, large airway collapse (LAC) refers to the excessive inward movement of the trachea and/or main bronchi during expiration. Aetiology of LAC includes tracheobronchomalacia and excessive dynamic airway collapse (EDAC). Importantly LAC may be present in up to a third of patients with severe asthma (149) and can present with overlapping symptoms also seen in VCD and dysfunctional breathing such as chronic 'barking' cough and exertional dyspnoea.(150) Presence of LAC was not specifically investigated in this study, but future studies are recommended to examine the relationship between large airway collapse, dysfunctional breathing, VCD/laryngeal dysfunction and chronic cough further, as well as the role of CPAP in acute rescue therapy of VCD. Intralaryngeal injection of botulinum toxin has also shown some promise for the treatment of severe refractory VCD, with effects lasting for up to 14 weeks. However, published studies of this intervention have been small and observational(130), and larger randomised controlled trials are required to determine efficacy.

Chapter Seven: Exploring the relationship between cough and bronchial provocation challenge testing.

7.1 Introduction

This chapter comprises a study of patients presenting for bronchial provocation testing with mannitol to the Alfred hospital lung function laboratory. Mannitol is a dry powder sugar which osmotically induces airway smooth muscle contraction in patients with airway hyperresponsiveness due to asthma. Mannitol can also provoke cough and laryngeal dysfunction (VCD).(128) However, it was not known whether it could be used to identify patients with laryngeal/cough hypersensitivity with other adjuncts such as questionnaires and cough counting measures. This current study aimed to identify patients with increased cough sensitivity to mannitol and used a cluster analysis approach to classify patients according to defining clinical characteristics.

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Laryngeal hypersensitivity and abnormal cough response during mannitol bronchoprovocation challenge

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Abstract

Background and objective: Inhalational challenge with dry mannitol powder may potentially induce cough by two mechanisms: airway bronchoconstriction or laryngeal irritation. This prospective observational study investigated laryngeal and bronchial components of cough induced by mannitol challenge.

Methods: We recruited consecutive patients referred for clinical mannitol challenge. The Newcastle Laryngeal Hypersensitivity Questionnaire (LHQ) was administered. Throughout testing, coughs were audio-recorded to derive a cough frequency index per time and dose of mannitol. Relationships between cough indices, laryngeal hypersensitivity and bronchial hyperresponsiveness (BHR) were examined. Participants were classified by cough characteristics with k-means cluster analysis.

Results: Of 90 patients who underwent challenge, 83 completed both the questionnaire and challenge. Cough frequency was greater in patients with abnormal laryngeal hypersensitivity ($p = 0.042$), but not in those with BHR. There was a moderate negative correlation between coughs per minute and laryngeal hypersensitivity score ($r = -0.315$, $p = 0.004$), with lower LHQ scores being abnormal. Cluster analysis identified an older, female-predominant cluster with higher cough frequency and laryngeal hypersensitivity, and a younger, gender-balanced cluster with lower cough frequency and normal laryngeal sensitivity.

Conclusion: Cough frequency during mannitol challenge in our cohort reflected laryngeal hypersensitivity rather than BHR. Laryngeal hypersensitivity was more often present among older female patients. With the incorporation of cough indices, mannitol challenge may be useful to test for laryngeal hypersensitivity as well as BHR.

KEYWORDS
asthma, bronchoprovocation test, cough, dyspnoea, laryngeal hypersensitivity questionnaire, mannitol challenge

INTRODUCTION

Chronic cough is a common complaint presenting to general practitioners and respiratory physicians, affecting up to 10% of all adults.[1] It is associated with significant negative psychosocial effects and reduced quality of life.[2] Chronic cough is heterogenous and may be related to extrathoracic (laryngeal) or intrathoracic (bronchial) airway hyperresponsiveness. Extrathoracic airway hyperresponsiveness (EAHR) has been defined as a hypersensitive laryngoconstrictor reflex.[3] Treatable traits within EAHR include rhinitis, viral infection and gastroesophageal reflux disease. Previously, up to 90% of chronic cough cases were reportedly related to asthma, postnasal drip and/or gastroesophageal reflux.[4]

Recently, a paradigm shift has occurred, proposing that the most common aetiology for chronic cough in adults is sensory afferent neuronal pathway dysfunction in the cough reflex arc,[5–7] so termed chronic cough hypersensitivity syndrome. Patients with EAHR may also manifest a wide range of respiratory symptoms, such as dyspnoea, dysphonia and globus. Thus, given the similar underlying pathophysiology, chronic cough sensitivity often overlaps with other laryngeal sensory dysfunction syndromes such as vocal cord dysfunction (also termed inducible laryngeal obstruction), *globus pharyngeus* and muscle tension dysphonia.[8,9]

Treatment-resistant asthma, with variable airflow obstruction caused by bronchial hyperresponsiveness (BHR) and eosinophilic airway inflammation, may also commonly present with refractory cough and may coexist with or be a misdiagnosis for chronic hypersensitive cough. 'Cough-variant asthma' has been used to describe patients with predominant cough and BHR. Objective clinical tools to aid accurate diagnosis are essential to target appropriate treatment.[10] These asthma treatments include inhaled corticosteroids for patients with asthma and eosinophilic airway inflammation, in contrast to treatments for chronic cough hypersensitivity such as speech pathology, anti-tussives or specific receptor antagonists aimed to reduce sensory hypersensitivity.

Dry mannitol powder acts as a hyperosmolar agent to induce airway smooth muscle contraction and is thus used for indirect bronchoprovocation testing with high diagnostic specificity for asthma.[11] Although over 80% of patients experience cough during testing, severe cough as an adverse effect is only reported in 1.3%.[12,13] Cough frequency during mannitol challenge has previously been found to be associated with asthma independent of bronchoconstriction, as well as to be associated with chronic cough.[12,14] Mannitol has been used to stimulate cough in cough sensitivity testing[15] and to provoke vocal cord dysfunction as a laryngeal provocation test.[16]

The aim of this study was to investigate the laryngeal and bronchial components of cough and to characterize patients with abnormal cough responses. We hypothesized that cough frequency during mannitol challenge would be more likely to be associated with laryngeal dysfunction than asthma. We designed this study to investigate the cough response during mannitol challenge as a marker of EAHR and compare this to cough response and BHR. We also hypothesized that cluster analysis could classify participants based on cough response to mannitol. We then aimed to evaluate the clinical relevance of this approach by examining for between-cluster differences.

SUMMARY AT A GLANCE

Cough commonly occurs during bronchoprovocation testing with mannitol. In this study, higher cough frequency in patients undergoing mannitol provocation was associated with laryngeal hypersensitivity, but not bronchial hyperresponsiveness. Greater cough frequency was found in older female patients. Mannitol provocation may be a useful test for laryngeal hypersensitivity.

METHODS

Study design and participants

Consecutive patients who were referred to our respiratory laboratory for bronchoprovocation testing with mannitol as part of their clinical assessment during the study period were eligible for inclusion. Patients were mostly referred by respiratory specialists, although some were also referred by general practitioners in the community. All-comers (not just those referred with chronic cough) were included to reduce referral bias. Written informed consent was obtained and the indication for testing documented.

Laryngeal hypersensitivity questionnaire

Patients completed the 14-item self-administered Newcastle Laryngeal Hypersensitivity Questionnaire (LHQ) which examined laryngeal dysfunction across three domains—obstruction, pain/thermal and irritation.[9] The questionnaire has discriminant validity between patients with laryngeal hypersensitivity and healthy controls with a cut-off score of *less than 17.1* for abnormal sensitivity.

Mannitol challenge

Patients were instructed to withhold relevant medications prior to attending their test. Baseline spirometry was performed prior to provocation testing. Patients with forced expiratory volume in 1 s (FEV_1) less than 1.5 L and/or 70% predicted at baseline were excluded. The mannitol challenge was completed following the manufacturer's recommendations (Aridol™; Pharmaxis, NSW, Australia). Patients were instructed to inspire the dry powder slowly, but sufficiently to spin the capsule within the provided inhaler in stepwise dosing increments following the first empty placebo capsule. FEV_1 was measured after each dose of mannitol. The test was ceased, if there was a 10% fall in FEV_1 between doses, a 15% drop in FEV_1 from baseline (PD_{15}), indicating BHR, or if a cumulative dose of 635 mg was reached. A forced vital capacity and full inspiratory manoeuvre was performed at the end of the challenge. Following mannitol challenge, short-acting bronchodilator was administered to all participants and spirometry performed to ensure FEV_1 had returned to within 90% of baseline FEV_1 prior to departure from clinic.

The dose–response slope as a continuous variable was also calculated by dividing the percentage final change in FEV_1 by the cumulative dose of mannitol administered.

Cough assessment

Coughs during provocation testing were recorded with an audio-recorder for subsequent manual counting. Coughs were counted by three of the authors (JW-YL, NS and TRT). Cough measurement was during the first 30 s after each dose of mannitol was inhaled, with consistent duration of cough measurement between patients and individual

TABLE 1 Baseline characteristics of patients

Baseline characteristics	Total ($N = 83$)	Laryngeal hypersensitivity ($n = 48$)	No laryngeal hypersensitivity ($n = 35$)	p-value
Age, mean (SD), years	46.4 (14.8)	45.9 (15.1)	47.1 (14.4)	0.71
BMI, mean (SD), kg/m^2	28.5 (6.7)	28 (6.3)	28 (7.4)	0.77
Caucasian ethnicity, *n* (%)	72 (87)	41 (85)	31 (89)	0.68
Gender, *n* (%)				
Female	51 (61.4)	32 (67)	19 (54)	0.25
Smoking status, *n* (%)				
Never	54 (65)	34 (70.8)	20 (57)	0.20
Ex-smoker	23 (34.9)	14 (29)	15 (43)	0.19
Current smoker	0 (0)			
Pack-years (if ex-smoker), mean SD	13 (13)	15.29 (13.7)	11 (13.6)	0.42
Pre-existing asthma diagnosis, *N* (%)	14 (15)	13 (27)	1 (2.9)	0.004**
Spirometry: baseline FEV_1 (L), mean (SD)	3.0 (0.92)	3.0 (0.9)	3.18 (0.94)	0.4
FEV_1 (% predicted)[a]	95.3 (14.2)	94.9 (14.2)	95.9 (14.3)	0.75

Abbreviations: FEV_1, forced expiratory volume in 1 s.
[a]Global Lung Function Initiative (GLI) reference equation.
**P < 0.01.

discrete coughs counted. Cough measurements are presented as total coughs, coughs per minute and coughs per 100 mg mannitol (cough dose ratio). 'C2' was defined as the dose of mannitol inducing two coughs and 'C5' was defined as the dose of mannitol causing five coughs. A cut-off score of 12 coughs per 100 mg mannitol was taken to indicate cough hypersensitivity.[14]

Statistical analysis

All statistical tests were performed using SPSS version 22 (IBM, Armonk, NY). Concentration of mannitol was also calculated by log-dose–response curves. Descriptive statistics are presented as proportions for categorical variables, means and SDs for normally distributed continuous variables or medians and interquartile ranges otherwise. Univariate comparisons between two groups were made by chi-square for categorical data and Student's *t*-tests for continuous data. Non-parametric data were analysed by the Mann–Whitney *U*-test. A *p*-value of <0.05 was considered statistically significant. Relationships between laryngeal dysfunction and cough frequency were examined using Pearson's correlation for continuous variables and Wilcoxon rank sum analysis for comparison between the two groups. Multivariable logistic regression analysis was performed to identify predictors of abnormal cough response.

A non-hierarchical cluster analysis was performed to classify the study population by three variables: bronchial provocation (as approximated by the dose–response slope), LHQ score and cough dose ratio (coughs per 100 mg mannitol). Continuous variables selected for cluster modelling were chosen a priori to best answer the study question by their contribution to characterize cough by both bronchial and laryngeal components. K-means cluster analysis was the principal clustering technique. Measurements were standardized using *z*-scores for continuous variables and/or log transformed if required. Between-cluster comparisons were analysed using one-way analysis of variance for parametric variables and chi-square test for categorical proportions. Analyses with different cluster solutions were performed to determine the robustness of the clustering algorithm, and to confirm the ideal number of clusters.

RESULTS

Study participants

Bronchoprovocation testing with mannitol was completed by 90 patients. Seven patients chose not to complete the Newcastle LHQ and were excluded from analysis. Suspected asthma was the reason for referral of 55 patients (66%). Of these, six had documented childhood asthma and 10 had a previous diagnosis of asthma. Therapies for asthma were being taken by 29 patients (35%). Of these, two patients were on inhaled corticosteroid only, 20 were on combination inhaled corticosteroid/long-acting beta-agonist, five were taking short-acting beta-agonist only, one patient was taking theophylline and one was taking a leukotriene antagonist. Three patients were referred with suspected 'sulphite allergy' and four were referred for recurrent lower respiratory tract infection or bronchitis. Chronic cough was the specified reason for the test in 17 patients (presumably to explore asthma as a cause for cough), three were referred for investigation of exertional dyspnoea and one patient was referred as part of a work-up for an application to the defence force.

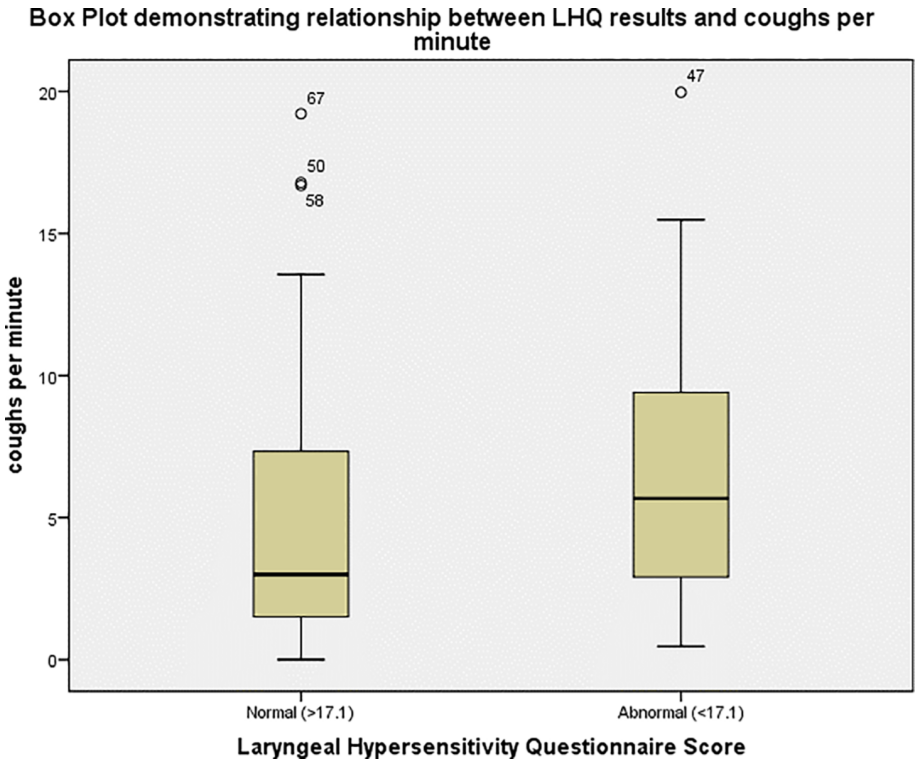

FIGURE 1 Boxplot demonstrating the relationship between laryngeal hypersensitivity questionnaire results and cough per minute index from mannitol challenge. Scores of 17 and above are consistent with no laryngeal hypersensitivity (normal score)[9]

Bronchial hyperresponsiveness

Overall, 18 patients (20%) had BHR as demonstrated by a PD_{15} of less than 635 mg. All patients who were referred for chronic cough or 'exertional dyspnoea' had a negative bronchoprovocation test. Of the 65 patients without demonstrable BHR, 34.3% were taking asthma medication.

Laryngeal hypersensitivity

The LHQ score was abnormal (<17.1) in 58% of patients. Patients with a pre-existing diagnosis of asthma were more likely to have laryngeal hypersensitivity ($p = 0.004$) (Table 1). There were no differences in other baseline clinical characteristics, as outlined in Table 1.

Cough analysis

Patients with abnormal LHQs had a higher cough per minute index compared to those who had normal laryngeal hypersensitivity (Mann–Whitney $U = 600$, median 5.7 [95% CI 3.4–6.8] vs. 3.0 [95% CI 1.9–5], $p = 0.042$) (Figure 1). However, patients with BHR did not have a significantly higher cough per minute index than those without BHR (median 5.2 [95% CI 3.4–8.2] vs. 4.2 [95% CI 4.6–7], $p = 0.78$). The Newcastle Laryngeal Hypersensitivity score was weakly negatively correlated with the total number of coughs ($r = -0.242$, $p = 0.029$) and coughs per minute ($r = -0.315$, $p = 0.004$), and was associated with C2 ($r = 0.256$, $p = 0.024$) but not C5 ($r = 0.219$, $p = 0.085$). There was also a weak negative correlation between Newcastle Laryngeal Hypersensitivity score and log (cough per 100 mg mannitol) ($r = -0.234$, $p = 0.038$). The relationship between abnormal cough response, laryngeal hypersensitivity and BHR is demonstrated in Figure 2.

Multivariable logistic regression was performed to identify predictors of an abnormal cough response (≥12 coughs per 100 mg mannitol)[14] during mannitol testing. Variables potentially affecting cough responses were included in the regression model: age, gender, smoking status, LHQ score, positive bronchial provocation test, BMI and FEV_1. The odds of having abnormal cough response were five times higher among females (OR 5.1, $p = 0.016$, 95% CI 1.4–19). With every increase in year of age, the odds of having abnormal cough response increased by 5.9% ($p = 0.009$, 95% CI 1.4–10.5). The odds of having an increased cough response were also higher among non-smokers compared to ex-smokers (OR 3.7, $p = 0.035$, 95% CI 1.1–13).

Multiple linear regression analysis performed with stepwise backward elimination for C2 and C5 identified that female gender was a significant predictor of these variables (beta coefficient for C2 = 122, 95% CI 62–181, $p < 0.001$; beta coefficient for C5 = 119, 95% CI 53–185, $p = 0.001$).

K-means cluster analysis was performed to classify the study population and confirm the results of the multivariable regression model. A two-cluster model best fitted the study data set. Two individuals were excluded—one was an outlier and one had incomplete data. Thus, 81 individuals were included in cluster analysis. Cluster 1 comprised patients with low (abnormal) LHQ score and a high cough

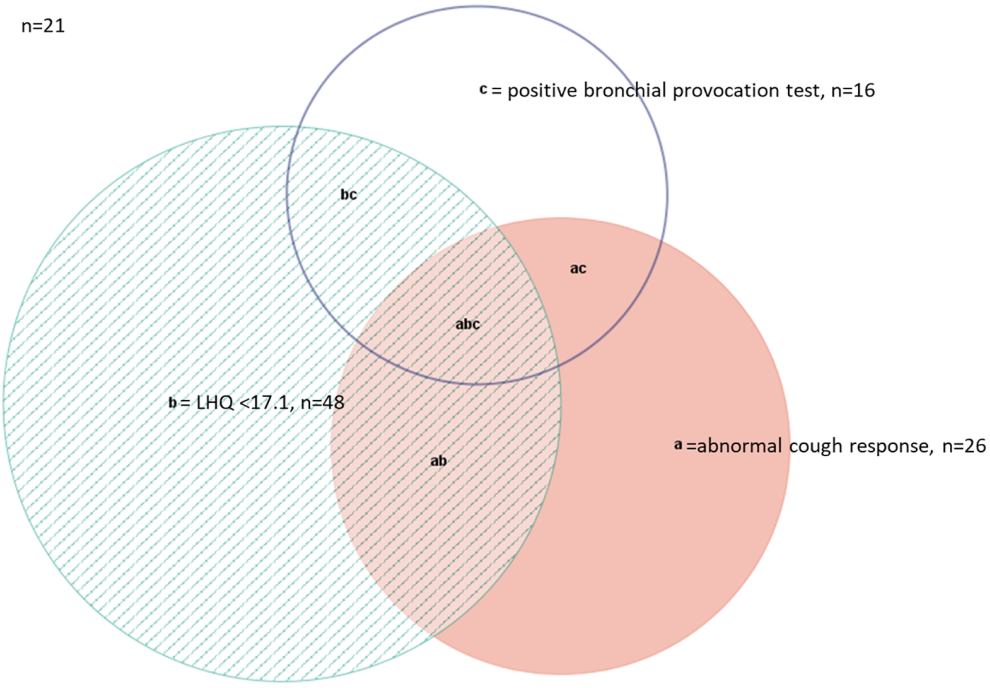

FIGURE 2 Venn diagram demonstrating the relationship between abnormal cough response, laryngeal hypersensitivity and bronchial hyperresponsiveness. a = cough >12/100 mg mannitol ($n = 26$), b = laryngeal hypersensitivity questionnaire <17.1 ($n = 48$), c = positive bronchial provocation test, $PD_{15} < 635$ mg ($n = 16$)

index, with significantly lower C2 and C5 values. Cluster 2 comprised patients with higher (normal) LHQ and low cough index, with significantly higher C2 and C5 scores. Bronchial responsiveness as measured by percentage fall in FEV_1 per milligram of mannitol inhaled was not a good discriminating variable for cluster membership (mean difference −0.012 [SE 0.016], 95% CI −0.044 to 0.02, $p = 0.448$). Cluster 1 included 26 cases and cluster 2 included 55 cases (Table 2 and Figure 3). Cluster 1 was a female predominant (96.2%), older group (mean age 51.6, SD 14.7), while cluster 2 comprised 47% females, with a mean age of 44 (SD 4.5) years.

DISCUSSION

In this clinical cohort undergoing mannitol bronchial provocation, increased cough response was associated with questionnaire-defined laryngeal hypersensitivity, but not BHR, suggesting that laryngeal dysfunction was the more common cause of cough hypersensitivity. Patients with laryngeal hypersensitivity were older and more likely to be female. With the incorporation of cough indices, inhaled mannitol may have the potential as a laryngeal as well as bronchial provocation agent.

In this cohort, an increased cough response (generally in the absence of BHR) was also associated with a pre-existing clinician diagnosis of asthma. A third of these patients were taking asthma medications for their symptoms, despite an absence of demonstrable variable airflow obstruction. However, it is also possible that the use of asthma medications may have attenuated BHR, especially if not withheld for sufficient time prior to the test. This finding highlights the importance of objective testing to confirm a clinical diagnosis of asthma. While not confirmed in this study, it is possible that some of these patients might have been better served by therapy for laryngeal hypersensitivity rather than for BHR.

Cluster analysis demonstrated two distinct patient groups. Those with higher cough frequencies were more likely to also have abnormal LHQ scores and tended to be older and female. They coughed two (C2) and five times (C5) at significantly lower doses of inhaled mannitol. Those with less cough had more normal LHQ scores and were younger with an almost equal balance between genders. Higher doses of mannitol were required to induce two (C2) or five (C5) coughs. These findings are consistent with previous data.[17]

Ex-smokers had less cough sensitivity compared to non-smokers, consistent with most, but not all, previous studies.[18,19]

Cough inhalation challenges have been previously performed using acidic agents (e.g., citric acid, acetic acid and tartaric acid) and other stimulants such as capsaicin.[20,21] However, widespread clinical use of these agents has been limited by the need for specialized equipment, general tolerability and uncertainty surrounding significance of results.[22] Mannitol is readily available in most lung function laboratories and reasonably well tolerated. Incorporating the use of automated cough counting into the process would even further improve efficiency.[23] Cough sensitivity to hypertonic saline has also been shown to be increased among patients with asthma unrelated to airway hyperresponsiveness.[24]

The addition of cough frequency measurement to bronchial provocation testing with mannitol may be useful to widen the scope of this test beyond the detection of BHR.

TABLE 2 Cluster analysis results

Variable	Population summary ($n = 81$)	Cluster 1 Low LHQ, high cough index ($N = 26$)	Cluster 2 High LHQ, low cough index ($N = 55$)	Significance (p-value)	Chi-square tests of independence	Mean difference [SE] (95% CI)
Gender female (%)	51 (63)	25 (96.2)	26 (47.3)	**<0.0001*****	$X^2 = 18.09$	
Age (years) (SD)	47.5 (15)	51.6 (14.7)	44 (4.5)	**0.034***		7.5 [1.6] (0.6 to 14.40)
Caucasian ethnicity (%)	70 (86.4)	23 (88.5)	47 (85.5)	0.712	$X^2 = 0.14$	
BMI (kg/m^2), mean (SD)	28.3 (6.6)	29 (6.72)	28 (6.5)	0.511		1.04 [1.6] (−2.09 to 4.16)
Smoking (ex) (%)	28 (34.6)	6 (23.1)	22 (40)	0.135	$X^2 = 2.24$	
Asthma medications (yes), n (%)	28 (34.6)	9 (34.6)	19 (34.5)	0.995	$X^2 = 0$	
Pre-existing diagnosis of asthma, n (%)	16 (19.5)	2 (7.7)	14 (25.5)	0.061	$X^2 = 3.51$	
FEV_1 (% predicted) (SD)	95.6 (12.7)	95.7 (11.4)	95.6 (13.4)	0.981		0.074 [3.05] (−6 to 6.15)
Newcastle LHQ score, mean (SD)[a]	16.1 (3.4)	13.8 (3.7)	17 (2.5)	**<0.001*****		−3.4 [0.81] (−5 to −1)
Cough dose ratio[a]	9.8 (9.1)	19.6 (9.3)	5.2 (3.8)	**<0.001*****		14.5 [1.45] (10.6 to 18.3)
Dose–response slope[a]	0.023 (0.07)	0.015 (0.016)	0.0272 (0.81)	0.448		−0.012 [0.016] (−0.044 to 0.02)
C2	65.7 (138)	20.6 (61.7)	87.4 (159)	**0.01****		−66.8 [25] (−117.2 to −16.4)
C5	84 (126)	24.6 (34)	121 (148)	**<0.001*****		−97 [25] (−147 to −46.7)

Abbreviations: FEV_1, forced expiratory volume in 1 s; LHQ, laryngeal hypersensitivity questionnaire. * P<0.05, ** P<0.01, *** P<0.001
[a]Used to define the clusters.

The addition of flexible nasoendoscopy to this test may also add further diagnostic value to visualize paradoxical vocal fold movement and identify vocal cord dysfunction.[16,25] Thus, with these simple adjuncts, patients with cough could potentially undergo a 'triple test' using a single provocation agent to investigate for three common causes: asthma, laryngeal hypersensitivity and vocal cord dysfunction. However, further validation of these techniques is needed before they are ready for introduction into clinical practice. With the advent of new receptor antagonists for chronic cough such as ATP receptor antagonists targeting neuronal hypersensitivity (P2X3),[26] objective measurement of cough frequency and sensitivity will be important to confirm this diagnosis and assess treatment response.

To our knowledge, this is the largest study to investigate the cough response to mannitol and the first to look at laryngeal hypersensitivity, as measured by the Newcastle LHQ. This adds to previously small pilot studies assessing mannitol testing for laryngeal hypersensitivity.

Our data have been obtained from a single centre with a large asthma and allergy service which may have led to referral bias and limit the generalizability of results. However, patients were referred both from the community (general practitioners) and by respiratory specialists. Further multicentre studies to improve external validity of results are recommended. We recognize that cough during mannitol inhalation may be influenced by breathing technique, and so asked study participants to inhale mannitol slowly, to limit the impact on the upper airway. It would

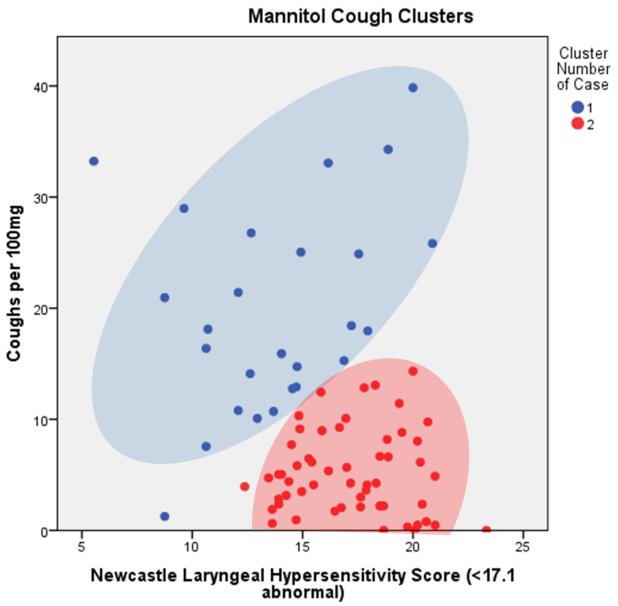

FIGURE 3 Graphical representation of mannitol cough clusters (blue dots, cluster 1; red dots, cluster 2)

have been ideal to obtain results from healthy subjects as controls, but previous studies suggest that excessive cough is uncommon during mannitol challenge among healthy subjects.[13] An elevated cough response is pathological, with differences in cough frequency when healthy subjects were compared to those with asthma and/or chronic cough.[22] Coughs were counted manually from near-patient audio recordings, rather than automated cough frequency monitoring. However, within pairs, close inter- and intra-observer agreement for cough counts have been previously reported.[27,28]

In conclusion, laryngeal hypersensitivity is an important cause of cough. Among patients presenting to our laboratory for bronchial provocation challenge testing, laryngeal hypersensitivity—rather than BHR—was a more common diagnosis identified during testing. Cough frequency during mannitol provocation may be a useful marker of laryngeal hypersensitivity.

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CONFLICT OF INTEREST

Michael J. Abramson holds investigator-initiated grants for unrelated research from Pfizer, Boehringer-Ingelheim and Sanofi. He has undertaken an unrelated consultancy for and received assistance with conference attendance from Sanofi. He has also received speaker's fee from GlaxoSmithKline. Mark Hew has received grants-in-aid, speaker fees and fees for serving on the advisory boards of GlaxoSmithKline, AstraZeneca, Novartis, Teva, Sanofi and Seqirus, all unrelated to the current manuscript, all paid to his institutional employer Alfred Health. Joy Wei-Yan Lee has received fees for providing unrelated independent medical advice for GlaxoSmithKline and has received speaker fees for medical education purposes from Boehringer Ingelheim, GlaxoSmithKline and AstraZeneca. The other authors have no conflicts of interest to declare.

AUTHOR CONTRIBUTIONS

Joy Wei-Yan Lee: Data curation (lead); formal analysis (lead); funding acquisition (lead); investigation (lead); methodology (lead); project administration (lead); writing – original draft (lead); writing – review and editing (equal). **Tunn Ren Tay:** Conceptualization (equal); data curation (equal); investigation (equal); writing – review and editing (supporting). **Brigitte M. Borg:** Data curation (supporting); investigation (supporting); resources (supporting); writing – review and editing (supporting). **Neha Sheriff:** Data curation (supporting); investigation (supporting); writing – review and editing (supporting). **Anne Vertigan:** Investigation (supporting); supervision (supporting); visualization (supporting); writing – review and editing (supporting). **Michael J. Abramson:** Conceptualization (equal); formal analysis (supporting); investigation (supporting); supervision (equal); writing – original draft (supporting); writing – review and editing (supporting). **Mark Hew:** Conceptualization (equal); formal analysis (equal); investigation (equal); methodology (equal); supervision (equal); writing – original draft (supporting); writing – review and editing (supporting).

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

HUMAN ETHICS APPROVAL DECLARATION

This study was performed in accordance with the Declaration of Helsinki. This human study was approved by Alfred Health Ethics Committee and Monash University Human Research Ethic Committee (approval: 464/15). All adult participants provided written informed consent to participate in this study.

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7.2 Summary of Findings from Chapter Seven

This study identified that the majority (80%) of patients presenting for bronchial provocation challenge test did not demonstrate airway hyperresponsiveness (AHR) defined as PD_{15} less than 635mg. About a third of patients without BHR were taking asthma medication. Laryngeal hypersensitivity was suspected to be present based on abnormal questionnaire scores in 58% of patients, and these patients had a higher cough per minute index during the challenge test. Predictors of an abnormal cough response during mannitol challenge testing included female gender, older age and non-smoking status. Cluster analysis identified two clusters of patients – one cluster with abnormal laryngeal sensitivity and higher cough indices, and another with normal laryngeal sensitivity and lower cough indices. Cluster one was female predominant (96.2%) and older (mean age 51.6 years) when compared to cluster two (47% female, mean age 44). The addition of cough frequency measurement to bronchial provocation testing with mannitol may be a useful adjunct to identify when laryngeal dysfunction with cough hypersensitivity is the dominant contributor to the patient's symptoms, rather than asthma or bronchial hyperresponsiveness.

As opposed to the other studies presented in this thesis, this study used a more general 'all-comer' population of patients, including those referred from primary care. This suggests that laryngeal hypersensitivity is highly prevalent, even outside of a difficult asthma patient population. It also means the findings of this study have a broader application, and should be of interest to not only respiratory physicians, but general practitioners in primary care as well. It confirms the importance of confirming variable airflow obstruction before escalating inhaled or even systemic steroids for patients with refractory cough.

Chapter Eight: Conclusions, Implications & Future Directions

8.1 Introduction and Summary of Novel Findings

This thesis has examined two major factors contributing to difficult-to-treat asthma: adherence to inhaled corticosteroid preventers and the common comorbidity of laryngeal dysfunction incorporating both vocal cord dysfunction (VCD) and cough hypersensitivity.

Despite the significant burden of difficult-to-treat asthma in Australia, little research had been done within these two areas specifically, with many knowledge gaps still present in the current literature.

The key and novel findings of this thesis are summarized below and will be subsequently discussed with respect to existing knowledge gaps and the five thesis aims as outlined in Chapter one.

1. *Medication adherence in difficult-to-treat asthma*

The first aim was to report the prevalence of poor adherence to inhaled corticosteroids among a sample of the Australian patient population with difficult asthma and to compare adherence measurement by electronic monitoring device (EMD) to subjective measures. This aim was addressed by the study presented in Chapter Three – *Non-adherence in the era of severe asthma biologics and thermoplasty* . In this study, 45 patients presenting to a tertiary difficult asthma centre underwent electronic monitoring (EMD) of inhaled preventers. Of these patients, 44.4% had confirmed non-adherence, as defined by less than 75% of prescribed doses. If patients who were suspected of poor adherence were also included, the rate of non-adherence increased to 55%. Many of these patients also had severe asthma and would have otherwise qualified for more advanced asthma treatments such as monoclonal biologics or bronchial thermoplasty. The proportion of patients with severe and difficult to treat asthma who were eligible for more expensive treatments such as biologics, yet remained poorly adherent to basic ICS preventer medication had not been previously described. Subjective measurement of adherence including self-reporting and

clinician impressions were inaccurate. This study identified how common non-adherence was in this patient population, even among those seen by specialists, and highlighted the importance of objective adherence measurement prior to escalation of treatment among patients with difficult-to-treat asthma, and that interventions to address and improve adherence in this patient group are urgently needed.

The second aim of this thesis was to use EMD data to develop metrics to describe medication adherence behaviour and to investigate whether these metrics can predict adverse asthma-related clinical outcomes. This was reported in Chapter Four – *Dynamics of inhaled corticosteroid use are associated with asthma attacks*. A novel finding of this chapter was the description of entropy as a surrogate measure of chaotic inhaler use, and how this could relate to significant clinical outcomes, including exacerbations. Entropy, as compared to usual averaged adherence measurements, reflected poorer asthma baseline quality of life and symptom control and was associated with an increased risk of asthma attacks requiring hospitalisation, GP visits or systemic corticosteroid use.

Taken together, these works highlight that medication non-adherence is a significant and prevalent issue within this patient population. Interventions to improve adherence include accurately measuring and using more sophisticated metrics such as Entropy. These techniques have the potential to assist clinicians more precisely identify patients at risk of poor asthma outcomes associated with non-adherence.

2. Laryngeal dysfunction

Laryngeal dysfunction has increasingly been recognized as a significant comorbidity to coexist with difficult-to-treat asthma.(16, 95) There has been a lack of agreement on a gold standard diagnostic process to objectively identify and distinguish between coexisting asthma and VCD. The clinical risk factors that predict the presence of VCD among patients with difficult-to-treat asthma have also not been previously well described and this was addressed in the studies outlined in Chapters Five (*Diagnosis of concomitant inducible laryngeal obstruction and asthma)* and Six (*Paradoxical vocal fold motion in difficult asthma is associated with dysfunctional breathing and preserved lung function)* of this thesis. Novel findings from Chapter Five included a description of a systematic assessment protocol for the diagnosis of inducible laryngeal obstruction (ILO/VCD) and distinguishing clinical features when assessing patients who also have suspected asthma. The findings from Chapter Five highlighted the importance of an objective process to accurately diagnose asthma when it is thought to coexist with ILO/VCD.

Novel findings from Chapter Six included identification of clinical features that were associated with the diagnosis of abnormal laryngeal function in a sample of an Australian population of patients with difficult-to-treat asthma. The novel findings in chapter six included the description of the predictive relationship between VCD and dysfunctional breathing, unrelated to airway inflammation or airflow obstruction, raising the possibility of shared pathogenesis between these two conditions. The presence of both VCD and dysfunctional breathing was likely to increase symptom burden and impact patient quality of life significantly.

The final aim of this thesis was to measure the cough response to bronchial provocation testing and test its' utility in identifying patients with laryngeal dysfunction or cough

hypersensitivity (as alternative explanations for the patients' symptoms, rather than asthma). This research question was addressed within Chapter Seven of this thesis – *Laryngeal hypersensitivity and abnormal cough response during mannitol bronchoprovocation challenge*. As provocation testing was not considered safe to undertake in a population of patients with severe asthma, a surrogate population of individuals presenting for challenge testing were studied. This study of 83 patients showed that the majority of those presenting for challenge testing did not demonstrate airway hyperresponsiveness (AHR). The study also identified clinical clusters of patients that were more likely to have an abnormal cough response including female gender and older age. The addition of cough frequency measurement and identification of laryngeal dysfunction to mannitol bronchial provocation testing was useful to assist with diagnosing laryngeal dysfunction as a possible cause of the patient's presenting symptoms other than AHR.

The rest of this chapter discussed these key findings and how they could be applied into clinical practice within the Australian health system. The strengths and limitations of the included research studies are also discussed, as well as suggested directions for future research.

8.2 Strengths of the Thesis

In addition to the novel contributions to the literature on difficult-to-treat asthma above, strengths of this thesis include the use of 'real world' data from clinical patients, the use of objective measures wherever possible, and prospective and robust data collection which may be easily replicated. Real world studies including 'all-comers' – reflect a more clinically diverse patient population compared to patients who have been recruited via a clinical trial

due to the absence of multiple strict exclusion criteria. This recruitment strategy also improves the external validity and generalizability of results which are likely to be applicable to other Australian tertiary hospitals.

Another strength of this thesis was the variety of questionnaires used for data collection. These questionnaires were not only used to assess asthma control and quality of life, but also to identify a variety of extra-pulmonary comorbidities. While previous approaches to diagnosis of comorbidities in difficult-to-treat asthma may have relied on clinical assessment or multiple investigations, the use of standardized questionnaires prior to protocol visits allowed for increased detection and targeted investigation. A previous study from the same cohort highlighted that these questionnaires significantly increased diagnostic yield of comorbidities, especially dysfunctional breathing (29.8% vs 4.8%, $p<0.001$), vocal cord dysfunction (33.3% vs 8.3%, $p<0.001$), sinonasal disease (79.8% vs 54.8%, $p<0.01$) and obstructive sleep apnoea (33.3% vs 14.3%, $p=0.005$).(92) The ideal questionnaire set to use clinically and for research purposes would contain questions that are easy to understand, well validated with reliable sensitivity, specificity and positive predictive values. If possible a minimal clinically important difference would be known so change with time could be interpreted. The questionnaires selected for use in this thesis mostly fulfilled these criteria where possible.

While self-reported data can be useful, both under and over reporting may be common issues, particularly with regards to medication adherence, cough frequency or symptom perception. A strength of the works included in this thesis was the use of measured, objective data wherever possible. This included the use of electronic monitoring devices to measure inhaler use, flexible nasoendoscopy and laryngoscopy to diagnose paradoxical vocal cord movement, and auditory recording and manual counting of cough frequencies.

This also means that these studies can be replicated using the same measurements and techniques among other patient populations.

Data capture for studies one, two, three and four were also undertaken using RedCAP – Research Electronic Data Capture. Therefore, thorough audit trails of all project activity were maintained and these custom-built databases may be shared with other collaborators for replication of results or future research projects.

Interstate and international research collaboration is also a strength of this thesis. The works in this thesis included collaboration with co-authors from the Woolcock institute in Sydney, New South Wales (study two), Changi General Hospital, Singapore (studies one, three, four and five) and John Hunter Hospital, Newcastle (study five). Collaboration also occurred with multiple disciplines including respiratory physicians, ear nose and throat specialists, respiratory nurses, speech language therapists and physiotherapists. These co-authors provided various levels of knowledge, specialty insights and experience to the included published works.

While there are well-established difficult asthma networks, for example, in the United Kingdom and other parts of Europe, the concept of the difficult asthma clinic and process is still relatively in its infancy in Australia and not available in all geographic areas, particularly in regional areas. The studies included within this thesis have studied local populations and thus contribute much needed data pertaining to the local Australian hospital outpatient clinic environment.

8.3 Limitations

Acknowledgement of the limitations of the works included are also important to consider when appraising the overall impacts of this thesis.

While data were prospectively collected, the studies were observational and sometimes limited to what data were available from routine clinical care. Observational data meant I was limited to reporting inference of associations, rather than concluding causation or definite predictors. However, temporality from repeated measures was able to be demonstrated. For example, follow up in study four was close to 100%.

While a strength of the thesis was the variety of questionnaires used, they did have limitations. Some patients were unable to complete the questionnaires, particularly if they were from a cultural and linguistically diverse background or had poor literacy. The number of questionnaires used in some of the studies could take up to forty minutes to complete for each patient. If a patient missed one question of the questionnaire, it meant that the total summary score was not available. Also while sensitivity and specificity were high for many questionnaires used, others, such as the GERD-Q for gastroesophageal reflux disease (151) had a sensitivity of 65% and specificity of 71%, potentially missing some diagnoses.

Regarding the questionnaires used for the middle airway clinic cohort, the Pittsburgh VCD Index(110) had a sensitivity of 83% and specificity of 95% for diagnosis of VCD, however the questionnaire was not designed for patients with concomitant VCD and asthma. The VCDQ was also developed as a symptom monitoring tool, rather than a diagnostic tool and included patients with VCD, VCD and asthma and healthy controls.(111) The dyspnoea index questionnaire was not specific for VCD, and the validation group included patients with vocal cord paralysis and laryngeal stenoses.(112) The Newcastle laryngeal hypersensitivity questionnaire used for studies reported in Chapters 5 and 7 included patients with a spectrum of dysfunctional laryngeal conditions including chronic cough, VCD, *globus pharyngeus* and muscle tension dysphonia.(113)

With respect to studies one and two, while the use of electronic monitoring data (EMD) was an objective measurement, there were inconsistencies in data collection due to issues

with device malfunction, disruption of transfer of data or patient interference (e.g. removal of device from the inhaler). In study one, this affected 20% of patients given an EMD – 5% due to device malfunction and 15% due to device reported lost or stolen. In study two, among the 68 patients who underwent inhaler monitoring, 16% had devices with missing data and 6% of devices malfunctioned. These exclusions contributed to the smaller numbers of patients included in final analyses, although useful statistically significant results were still obtained. These study sizes were also smaller due to the constraints in time for recruitment, and this could mean that some studies were underpowered to detect other clinically important differences as statistically significant .

The studies included in this thesis were also limited to a single tertiary hospital population, which had a large asthma and allergy service. This may affect the generalizability of the results when considering smaller secondary hospitals with less specialized services. Given the observational nature of the included studies, another acknowledged limitation is a lack of a control group, which would be an important consideration for future studies as described below in section 8.5.

8.4 Implications for Clinical Practice and Potential Applications Within the Australian Healthcare System.

This thesis has made significant contributions to the literature on factors contributing to difficult asthma, particularly within the areas of non-adherence to medication and laryngeal dysfunction as comorbidities of difficult to treat asthma. This section of the discussion will include suggestions on how these novel findings may be used in clinical practice and how they may be applied within the greater Australian healthcare system, either now, or in the future.

1. *Medication adherence and inhaler monitoring*

Studies one and two of this thesis have highlighted the importance of objective inhaler monitoring among this patient population and that clinical detection of nonadherence is unreliable. This is not currently widely undertaken in general practice nor even general respiratory outpatient clinics. Current barriers to this include: the cost of devices, time required to fit the device and/or download the data and the multiple types of inhalers available. Integration of adherence monitoring into inhalers themselves at the point of manufacture is a potential way forward to overcome these barriers and could be a key component to optimized and personalised asthma management in the future.(58) Objective adherence monitoring has already been implemented in the field of sleep medicine, such as during assessment of patients with severe obstructive sleep apnoea for subsidized continuous positive airway pressure machines (CPAP). Based on research from this thesis, the clinic at Alfred health continues to fit these monitoring devices for patients presenting for specialized difficult asthma assessment if they have a compatible inhaler. Biologics for severe asthma can cost between $450 to more than $1600 per month per patient. The Pharmaceutical Benefits Scheme (PBS), which subsidizes biological agents for severe asthma, requires that the patient has received optimized asthma therapies including adherence to high dose inhaled corticosteroid and long-acting beta-2 agonist therapy for at least twelve months, prior to accepting an application for the biologic. However, it is not currently specified how this adherence is demonstrated. The findings from this thesis suggest that confirmation of adherence with objective measurement is preferable and perhaps should be required.

The use of entropy as a calculated measure for adherence may be introduced into the asthma clinic if adherence monitors integrated into inhalers became mainstream.

There exists potential to develop an online calculator or tool with an automated interpretation guide which could be used by clinicians to identify patients at risk of worse asthma outcomes. Entropy measurements may also prompt the clinician to evaluate or screen the patient for other features of life chaos, such as mental health disorders, socioeconomic disadvantage or substance abuse.

2. *Laryngeal dysfunction*

Laryngeal dysfunction is common yet under-recognised and often misdiagnosed, and patients may be treated inappropriately with escalating asthma therapies. The results from study three of this thesis could be used by other hospitals as a guide for how to conduct a multidisciplinary and systematic 'middle airway' clinic assessment protocol to guide diagnosis and management of coexisting VCD and asthma. Given the defined protocol and existing database that is available to be shared via RedCAP(141), it would be possible even for non-respiratory physicians or ENT surgeons, or general physicians in more rural areas, to implement these diagnostic tools. Telehealth for a multidisciplinary discussion of patients with tertiary centres, may also help to implement these clinics and allow specialized services for patients living at a greater distance from a tertiary centre to be more available. The benefits of incorporating this protocol into a standard of care include ceasing unnecessary asthma treatments, educating patients to interpret their symptoms and directing patients towards interventions that are more likely to be effective for relief of symptoms, e.g. rescue breathing strategies, reducing the risk of healthcare utilization.

The results from study four highlighted the importance of identifying and treating dysfunctional breathing (DB) alongside vocal cord dysfunction among patients with difficult to treat asthma, particularly if lung function is normal. Taking steps to examine

for the presence of these diagnoses, for example: with validated questionnaires, clinical history and objective measures such as laryngoscopy, should be included in difficult asthma treatment guidelines. Involving a respiratory physiotherapist with an interest in respiratory retraining as well as speech language therapy for management of laryngeal dysfunction is also likely to be essential to improve patient outcomes.

Lung function laboratories could consider incorporation of cough measurement as a part of bronchial challenge testing to help identify patients with abnormal laryngeal hypersensitivity, particularly if the bronchial provocation component is negative. Automating cough counting by recording device, would make this more convenient and feasible.(153)

3. *Integration of thesis findings within a treatable traits approach*

A treatable traits model of care was first proposed in 2016 as a targeted and precision medicine approach for patients with obstructive airways disease, including asthma.(21) A treatable trait can be defined as a 'therapeutic target identified by phenotype or endotypes through a validated biomarker'(154). Consensus expert opinion has agreed on the three key attributes of a treatable trait, including that they are:

1. Clinically important
2. Recognisable and measurable and
3. Treatable (i.e. responsive to treatment).(155)

Further research is underway to understand the best way to translate this new paradigm into clinical practice, however a multidimensional assessment to identify these traits, essentially the systematic assessment undertaken in the clinical studies reported here, is a key component. Patients are thus assessed for a specified set of treatable traits and an individualized treatment strategy is proposed based on the identification of these traits.(156)

In asthma, treatable traits can be divided further into pulmonary, extrapulmonary and behavioural/risk factors domains, and over 50 candidate traits have been identified.(90, 156) Medication non-adherence is considered a behavioural/risk factor treatable trait, cough reflex hypersensitivity a pulmonary treatable trait and VCD an extrapulmonary treatable trait. The Centre of Excellence in Treatable Traits recently highlighted the top five treatable traits in obstructive airways disease to prioritize at its annual research meeting to include:

1. Non-adherence
2. Obesity
3. Physical activity and sedentary behaviour
4. Anxiety and depression
5. Type two airway inflammation and vocal cord dysfunction (equivalently ranked 5th).

(Personal communication, May 2022, Newcastle, NSW, Australia)

Therefore, the findings of this thesis can easily be applied within a treatable traits paradigm. The Treatable Traits Down Under International Workshop Report identified key outstanding research questions including prevalence of individual traits in different populations (*studies one, three and five of this thesis)*, which treatable traits matter *(study 2)*, what is the best treatment for specific traits *(study four)* and how best to implement a treatable traits approach *(study three).*(156)

8.5 Future Directions

The key works from this thesis provide a foundation for ongoing research and have raised future research questions. Some of these studies have been commenced or undertaken at Alfred health in similar patient populations.

1. *Medication adherence and inhaler monitoring*

A focus for future research includes improved inhaler monitoring and evaluation, and while this may be a task for the device industry, development should be conducted in conjunction with key stakeholders such as patients and their treating physicians. Some asthma inhaled medication may require to be reformulated and additional clinical trials may be requested by the Therapeutics Goods Administration.

A subsequent study planned for beyond this thesis includes a discrete choice experiment (DCE) to investigate EMD inhaler preferences in health care providers and patients with asthma. With current focus on patient-centered healthcare, there is a need to understand how patients value the different attributes of the EMD to ensure future engagement. DCEs can be used as a patient-preference analysis to inform policy. Understanding how patients and their health care providers decide about how they use EMDs will be key to improving integration of this technology into routine asthma care.

With regards to measurement of entropy calculations, further research is currently being undertaken to replicate this study in other asthma populations, such as among patients with mild-moderate asthma, and patients with asthma treated in primary care. Further studies could also explore the impact of comorbidities such as mental health disorders, substance abuse, or other factors such as patient socioeconomic factors or device polypharmacy on the entropy metric and inhaler adherence behaviour. Furthermore, it would be useful for future studies to focus on developing an evidence-based approach for the management of a patient who demonstrates high entropy on inhaler adherence assessment. Possible interventions could include repeated and

personalized counselling and education with health care professionals (this could be provided remotely via telehealth), incentives to promote adherence (such as an electronic rewards or points system and prescription cost subsidy program) and inhaler taking reminders.(152)

2. *Laryngeal dysfunction*

Following on from the works published in this thesis, a further study was published in a larger population of patients (*Other publications during candidature)* investigating the diagnostic and therapeutic outcomes following systematic assessment of patients with concomitant vocal cord dysfunction and asthma. On this occasion, clinician assessment and laryngeal hypersensitivity questionnaire results predicted the presence of VCD and de-escalation or cessation of asthma inhaled therapy was possible in 63% of patients without variable airflow obstruction.(157) There was also subjective improvement in VCD following speech therapy. I am also currently undertaking a randomised clinical trial in this patient population in conjunction with the speech therapy department looking at the effectiveness of speech therapy ± amitriptyline for patients with VCD.

Other future research in progress in this area include a Delphi survey, a framework based on rounds of questioning to a panel of experts to generate a consensus expert opinion on how clinicians diagnose VCD. The round is underway, assessing clinical scenarios and the second round consisting of statement ratings will be conducted in 2022.

Other future research projects ongoing at my campus include validation of diagnostic laryngoscopy tests using mannitol (comparing with a control population of normal volunteers) and odour provocation to assist with the diagnosis of trigger-induced VCD. I also plan to commence continuous laryngoscopy with exercise via cardiopulmonary

exercise testing with further opportunity to conduct research studies regarding the robustness of this test to confirm the diagnosis and severity of exercise-induced VCD/ILO.

8.6 Closing Remarks

Inhaler adherence behaviour and laryngeal dysfunction as factors contributing to difficult-to-treat asthma have been extensively investigated in the research presented. Taken together, the five aims and objectives of this thesis were met by the five research studies included. In the first part of this thesis, the prevalence of non-adherence in a difficult-to-treat asthma population was examined and was found to affect a significant proportion of patients, even among those presenting for specialist care. Secondly, entropy metrics as a measure of non-adherent patterns of inhaler use were explored. In the second section of this thesis, three studies examined the important comorbidity of laryngeal dysfunction when it coexisted or masqueraded as asthma. This included examining the clinical predictors of VCD/ILO, a systematic assessment protocol for objective diagnosis and identifying cough hypersensitivity among patients presenting for bronchial provocation testing. The findings from the works presented here have identified targets for future research, but may now also be applied in practical ways to improve the care for patients with difficult-to-treat asthma.

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Appendix

Ethics Approval Certificates

TheAlfred

ETHICS COMMITTEE CERTIFICATE OF APPROVAL

This is to certify that

Project No: 285/15

Project Title: Clinical Audit of Difficult Asthma Clinic

Principal Researcher: A/Professor Mark Hew

was considered for Low Risk Review and ***APPROVED*** *on* **25/06/2015**

It is the Principal Researcher's responsibility to ensure that all researchers associated with this project are aware of the conditions of approval and which documents have been approved.

The Principal Researcher is required to notify the Secretary of the Ethics Committee, via amendment or report, of

- Any significant change to the project and the reason for that change, including an indication of ethical implications (if any);
- Serious adverse effects on participants and the action taken to address those effects;
- Any other unforeseen events or unexpected developments that merit notification;
- The inability of the Principal Researcher to continue in that role, or any other change in research personnel involved in the project;
- A delay of more than 12 months in the commencement of the project; and,
- Termination or closure of the project.

Additionally, the Principal Researcher is required to submit:

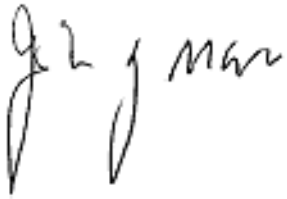
- A Final Report on completion of the project.

Approval covers the project as described in the application (including any modifications made prior to approval). Low Risk projects are subject to audit and ethical approval may be withdrawn if the project deviates from that proposed and approved.

SPECIAL CONDITIONS

None

SIGNED:



Professor John J. McNeil
Chair, Ethics Committee

Please quote project number and title in all correspondence

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Ethics Committee

Certificate of Approval of Amendments

This is to certify that amendments to

Project: **37/16 Clinical audit of the upper AIRMED Upper Airway clinic**

Principal Researcher: **A/Professor Mark Hew**

Amendment: **Change to research personnel**
Principal Investigator is now A/Professor Mark Hew'
Dr Eve Denton and Dr Joy Lee appointed as co-researchers.
Dr Tunn Ren Tay has left the study

have been approved in accordance with your amendment application dated **18-May-2018** on the understanding that you observe the National Statement on Ethical Conduct in Human Research.

It is now your responsibility to ensure that all people associated with this particular research project are made aware of what has actually been approved and any caveats specified in correspondence with the Ethics Committee. Any further change to the application which is likely to have a significant impact on the ethical considerations of this project will require approval from the Ethics Committee.

Professor John J. McNeil
Chair, Ethics Committee

Date: **1-Jun-2018**

All research subject to Alfred Hospital Ethics Committee review must be conducted in accordance with the National Statement on Ethical Conduct in Human Research (2007).

The Alfred Ethics Committee is a properly constituted Human Research Ethics Committee operating in accordance with the National Statement on Ethical Conduct in Human Research (2007).

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ETHICS COMMITTEE CERTIFICATE OF APPROVAL

This is to certify that

Project No: 37/16

Project Title: Clinical audit of the upper AIRMED Upper Airway clinic

Principal Researcher: Dr Tunn Ren Tay

was considered for Low Risk Review and ***APPROVED*** *on* **18/01/2016**

It is the Principal Researcher's responsibility to ensure that all researchers associated with this project are aware of the conditions of approval and which documents have been approved.

The Principal Researcher is required to notify the Secretary of the Ethics Committee, via amendment or report, of

- Any significant change to the project and the reason for that change, including an indication of ethical implications (if any);
- Serious adverse effects on participants and the action taken to address those effects;
- Any other unforeseen events or unexpected developments that merit notification;
- The inability of the Principal Researcher to continue in that role, or any other change in research personnel involved in the project;
- A delay of more than 12 months in the commencement of the project; and,
- Termination or closure of the project.

Additionally, the Principal Researcher is required to submit

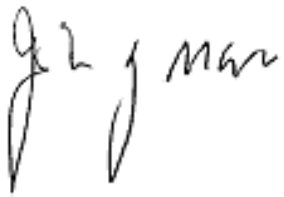
- A Final Report on completion of the project.

Approval covers the project as described in the application (including any modifications made prior to approval). Low Risk projects are subject to audit and ethical approval may be withdrawn if the project deviates from that proposed and approved.

SPECIAL CONDITIONS

None

SIGNED:



Professor John J. McNeil
Chair, Ethics Committee

Please quote project number and title in all correspondence

Monash University Human Research Ethics Committee

Confirmation of Registration

Project Number: 14655
Project Title: Clinical audit of the AIRMED upper airway clinic
Chief Investigator: Assoc Professor Mark How
Registration Date: 18/07/2018
Expiry Date: 18/07/2023

Terms:

1. Registration is valid whilst you hold a position at Monash University, and approval at the primary HREC is current.
2. This notification does not constitute HREC approval. It is the responsibility of the Chief Investigator to ensure that approval from the primary HREC continues for the duration of the research.
3. End of project: You should notify MUHREC at the conclusion of the project or if the project is discontinued before the expected date of completion.
4. Retention and storage of data: The Chief Investigator is responsible for the storage and retention of the original data pertaining to this project in accordance with the *Australian Code for the Responsible Conduct of Research*.

Kind Regards

Professor Nip Thomson

Chair, MUHREC

CC: Dr Joy Lee, Ms Eve Denton

TheAlfred

Ethics Committee

Certificate of Approval of Amendments

This is to certify that amendments to

Project: **464/15 The association between cough and bronchoconstriction during mannitol challenge**

Principal Researcher: **Dr Joy Lee**

Amendment: **Change to research personnel**
Departure of Dr Tunn Ren Tay
Appointment of Dr Joy Lee as Principal Investigator
PICF Version 3, 13-05-2016

have been approved in accordance with your amendment application dated **1-Mar-2016** on the understanding that you observe the National Statement on Ethical Conduct in Human Research.

It is now your responsibility to ensure that all people associated with this particular research project are made aware of what has actually been approved and any caveats specified in correspondence with the Ethics Committee. Any further change to the application which is likely to have a significant impact on the ethical considerations of this project will require approval from the Ethics Committee.

Professor John J. McNeil
Chair, Ethics Committee

Date: **19-May-2016**

All research subject to Alfred Hospital Ethics Committee review must be conducted in accordance with the National Statement on Ethical Conduct in Human Research (2007).

The Alfred Ethics Committee is a properly constituted Human Research Ethics Committee operating in accordance with the National Statement on Ethical Conduct in Human Research (2007).

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ETHICS COMMITTEE CERTIFICATE OF APPROVAL

This is to certify that

Project No: 464/15

Project Title: The association between cough and bronchoconstriction during mannitol challenge

Principal Researcher: Dr Tunn Ren Tay

was considered for Low Risk Review and ***APPROVED*** *on* **05/10/2015**

It is the Principal Researcher's responsibility to ensure that all researchers associated with this project are aware of the conditions of approval and which documents have been approved.

The Principal Researcher is required to notify the Secretary of the Ethics Committee, via amendment or report, of

- Any significant change to the project and the reason for that change, including an indication of ethical implications (if any);
- Serious adverse effects on participants and the action taken to address those effects;
- Any other unforeseen events or unexpected developments that merit notification;
- The inability of the Principal Researcher to continue in that role, or any other change in research personnel involved in the project;
- A delay of more than 12 months in the commencement of the project; and,
- Termination or closure of the project.

Additionally, the Principal Researcher is required to submit

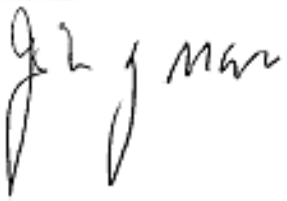
- A Final Report on completion of the project.

Approval covers the project as described in the application (including any modifications made prior to approval). Low Risk projects are subject to audit and ethical approval may be withdrawn if the project deviates from that proposed and approved.

SPECIAL CONDITIONS

None

SIGNED:



Professor John J. McNeil
Chair, Ethics Committee

Please quote project number and title in all correspondence