DOCTOR OF MEDICINE

Role of long acting bronchodilators in asthma and chronic obstructive pulmonary disease

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Table of Contents:

Contents

List of Figures:

List of Tables:

List of Abbreviation:

6MWT: Six-minute walk test

ACEI: Angiotensin converting enzyme inhibitor

ACQ: Asthma control questionnaire

AHR: Airway hyperresponsiveness

ANOVA: Analysis of variance

ARB: Angiotensin receptor blocker

AX: Reactance area under the curve

β-ADR: Beta-adrenoceptor

BDI: Baseline dyspnoea index

BMI: Body mass index

BP: Blood pressure

CI: Confidence interval

COPD: Chronic obstructive pulmonary disease

CONSORT Consolidated Standards of Reporting Trials

DBP: Diastolic blood pressure

DPI: Dry powder inhaler

Eos: Eosinophils

ECP: Eosinophilic cationic protein

FEF25-75 Forced expiratory flow between 25-75% of FVC

FeNO: Fractional exhaled nitric oxide

FEV_1: Forced expiratory volume in 1 second

Fres: Resonant frequency

FVC: Forced vital capacity

GCP: Good clinical practice

GINA Global Initiative for Asthma

GOLD: Global Initiative for chronic obstructive lung disease

HF: Heart failure

HR: Heart rate

ICS: Inhaled corticosteroid

IgE: Immunoglobulin E

IL: Interleukin

IMP: Investigational medicinal product

IOS: Impulse oscillometry

LABA: Long acting beta-2 receptor agonist

LAMA: Long acting muscarinic receptor agonist

MCID: Minimal clinically important difference

MHRA: Medicines and Healthcare Regulatory Authority

NHS: National Health Service

NYHA: New York heart association

PD_{20}: Provocative dose causing 20% fall in FEV1

PEF: Peak expiratory flow

PPB: Parts per billion

R5: Resistance at 5Hz

R20: Resistance at 20Hz

REC: Research Ethics Committee

RVC: Relaxed vital capacity

SABA: Short acting beta-2 agonist

SCRR: Scottish Centre for Respiratory Research

Sp02: oxygen saturation

SBP: Systolic blood pressure

SGRQ: St George's Respiratory Questionnaire

TDI: Transition dyspnoea index

X: Lung reactance

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This thesis is dedicated to my wife, Dr Lucy Doogan, for her unreserved love and support.

Declaration:

I (Dr Sunny Jabbal) hereby declare that I am the author of this thesis. All the references cited have been consulted by the author. All the data was collected by the author, or on behalf of the author by staff at the Scottish Centre of Respiratory Research. All works within this thesis are published in peer reviewed journals and I confirm I have first authorship on those referenced works. I was responsible for all statistical analysis of the data published within this thesis.

I declare that the work described in this thesis has not been previously submitted for a higher degree. The work contained in this thesis was carried out during my position as a Clinical Research Fellow (both substantive and honorary) in the Division of Molecular and Clinical Medicine, Ninewells Hospital & Medical School, University of Dundee between August 2015 and August 2019.

Signed:

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Summary Statement:

Long acting beta agonists (LABA) and long acting muscarinic receptor antagonists (LAMA) are both used in the treatment of obstructive airway disease, in asthma they are given with inhaled corticosteroids (ICS), in chronic obstructive pulmonary disease (COPD) they may be given with or without ICS. These therapies may attenuate the degree to which airways narrow to a bronchoconstrictor stimulus (airway hyperresponsiveness). Long acting bronchodilators may also change airway geometry as well as pure airway calibre, this can be measured by an effort independent breathing test called impulse oscillometry. The works within this thesis assess the effects of LABA and LABA with LAMA therapy on the airways of non-smoking people with asthma, people with asthma who smoke, and people with COPD. Primarily it focuses on outcomes related to impulse oscillometry measurements of airway resistance (R) and reactance (X), but also assesses how the bronchodilators attenuate airway hyperresponsiveness.

In mild-moderate persistent asthma, the addition of the LAMA tiotropium to ICS/LABA adds little to airway calibre or geometry. Neither does it significantly attenuate airway hyperresponsiveness. In the group of smokers with asthma, the addition of tiotropium to ICS/LABA does significantly reduce airway resistance and reactance, alongside asthma symptoms. In COPD tiotropium added to ICS/LABA reduces bronchoconstriction from non-selective beta-blocker therapy with carvedilol.

In summary add on LAMA to ICS/LABA has little benefit in mild-moderate asthma but may offer benefits in both people with asthma who smoke and those with COPD. This may be due to a different inflammatory or bronchoconstrictor stimulus in those patients.

Introduction

Objective:

The objective of this thesis was to compare three airway disease or “responder” groups:

- Non-smoking asthma
- Smoking-asthma
- Ex-smoking chronic obstructive pulmonary disease (COPD)

The main effect measured was the response to (or impact of) two classes of long acting bronchodilators primarily on pulmonary function outcomes. The bronchodilators used were a long acting beta agonist (LABA) and a long acting muscarinic receptor antagonist (LAMA). This thesis comprises three published clinical trials.

The overarching hypothesis was that these three disease groups respond to a differing degree to long acting bronchodilator therapy added onto inhaled corticosteroid (ICS). One of the key mechanisms thought to explain why the groups may respond differently was varying degrees of airway hyperresponsiveness (AHR). This was assessed by exposing the groups to bronchoconstrictor stimuli and measuring how much protection the bronchodilators conferred to each of the responder groups.

Definitions in airway disease:

The decision to impose strict definitions between asthma and COPD have always been a source of debate. The world's first international symposium on obstructive airway disease occurred in 1960. This "bronchitis" symposium introduced two key hypotheses as to the nature of asthma and chronic bronchitis: The "Dutch" hypothesis, by Orie and Sluiter (University of Groningen), and the so called "British" hypothesis by Fletcher and Pride (Hammersmith, London). The "Dutch" hypothesis proposed asthma and chronic bronchitis, hereafter referred to as chronic obstructive pulmonary disease (COPD), had common origin and clinical manifestations, with both being determined by endogenous (genetic, age, and sex) and exogenous (allergens, smoking, viruses, and air pollution) factors (1). The "British" hypothesis had the opposite view of totally differentiating these diseases from one another, suggesting no common origin or relationship between asthma and COPD (2). Whilst the debate still persists regarding which hypothesis has evolved to be true in the era of advanced immunology, imaging, and functional testing, one aspect of the Dutch hypothesis remains ever relevant, specifically that patients should be appropriately grouped by clinical characteristics, rather than a single disease label.

Pointedly, as recently as 2017, *The Lancet* Commission on airway disease recommends the deconstruction of the arbitrary labels of asthma and COPD into its component parts, with a focus on traits that are identifiable and treatable (3). These so-called "treatable traits" encompass both asthma and COPD and include airflow

limitation (fixed vs variable), infection, and eosinophilic airway inflammation among others (3). Treatable traits can predict risk of future asthma exacerbations (4).

Aside from common treatable traits the other commonality between the spectrum of asthma and COPD is this: Both diseases are manged pharmacologically with inhaled bronchodilator therapy in the form of long acting beta 2 agonist (LABA) and long acting muscarinic receptor antagonist (LAMA) with or without the presence of inhaled corticosteroids.

Outside of treatable traits, disease groupings in asthma and COPD can include phenotypes and endotypes (5). Phenotypes may be considered a precursor to the endotype, as they are an observable property of a disease based on clinical characteristics, triggers, or general inflammatory processes. Endotypes define a specific biological pathway to explain the observable property of a phenotype. Whilst a person's phenotype may evolve over time their endotypes do not change with time or treatment.

Endotypes in asthma include T-helper 2 (Th-2) or type 2 (T2) high and low categorisation. Endotypes in COPD include eosinophilic and non-eosinophilic COPD. Despite the evolving science of endotypes and commonalities in inhaled therapy, asthma and chronic obstructive pulmonary disease are still traditionally considered as distinct entities, with guidelines recommending different management approaches for each condition (6, 7). Asthma is defined as "a *heterogeneous disease, usually characterised by chronic airway inflammation. It is defined by the history of respiratory symptoms such as wheeze, shortness of breath, chest*

tightness and cough that vary over time and intensity, together with a variability of expiratory airflow limitation"(6). COPD is defined by the Global Initiative for Chronic Obstructive Lung Disease (GOLD) as a *"disease that is characterised by persistent respiratory symptoms and airflow limitation that is due to airway and/or alveolar abnormalities, usually caused by significant exposure to noxious particles or gases"* (7). Asthma and COPD are both heterogeneous conditions, which have overlapping physiological and pathological features, whilst also sharing similar pharmacotherapy of inhaled corticosteroids (ICS) long acting beta-2 agonist (LABA), and long acting muscarinic receptor antagonists (LAMA). Despite this, we arbitrarily divide their management based on disease label instead of their predominant treatable trait (3). The purpose of this thesis is to assess the efficacy of the inhaled therapy common to both asthma and COPD using novel mechanisms such as airway hyperresponsiveness and impulse oscillometry, further described below.

Pathophysiology:

To better understand how to approach airway disease, one must consider the predominant mechanisms of airway inflammation. On a pathophysiological level, airway inflammation can be divided into two endotypes, type 2 airway inflammation (associated with atopic and eosinophilic asthma) and non-type 2 airway inflammation (associated with non-atopic, non-eosinophilic asthma and COPD).

Type 2 airway inflammation:

Asthma is predominantly due to Type 2 (or T-helper 2 cell) derived airway inflammation. Upon exposure to a stimulus, such as a virus or allergen, airway epithelium releases cell cytokines, such as interleukin (IL) -33, and thymic stromal lymphopoietin (TSLP). IL-33 also promotes type-2 innate lymphoid cells (ILC2) to release large amounts of Type 2 cytokines (mainly IL-5 and IL-13) (8).

TSLP causes dendritic cells to mobilise to local lymph nodes. Dendritic cells activate naïve CD4 T cells, these cells now in an IL-4 competent (activated) state. These IL-4 competent (activated)T cells mature into T-follicular helper (TFH) cells and T-lymphocyte (TH) 2 cells. The TFH, a major producer of IL-4, promote B cell mediated class switching in lymph nodes. B cells are now switched on to Immunoglobulin E (IgE) production. Heterogeneity exists within IgE function (9) and IgE does not require cross linkage to exert its effect on mast cell function. IgE primes mast cells to release histamine, and basophils to release leukotrienes. One such leukotriene (D4) causes smooth muscle contraction, and mucus hypersecretion. TH2 cells migrate from lymph nodes to the airway submucosa and produce IL5 and IL13. Both these interleukins result in cascading airway inflammation. IL13, promoting airway hyperresponsiveness (by enhancing smooth muscle contraction), promotes airway fibrosis, and causes differentiation of epithelial cells into goblet cells with resultant mucus hypersecretion (10). Additionally, IL-13 independently induces transcription of inducible nitric oxide synthase (iNOS) from bronchial epithelial cells (10). Nitric oxide synthase (NOS) has two isoforms constitutive and inducible (iNOS), it is the former that has a modulatory role in asthma inflammation (11), and results in

synthesis of the free radical nitric oxide (NO) from L-arginine. NO is a gaseous signalling molecule that reacts with superoxide A, resulting in airway inflammation and an increase in collagen and elastic fibre deposition, causing subsequent airway remodelling (12). As NO is not stored, it is exhaled and can be quantitatively measured as a marker of eosinophilic airway inflammation. One method of measurement is termed fractional exhaled nitric oxide (FeNO) (13). FeNO may be used to assess treatment response in allergic airway inflammation (14), the former having been shown to be associated with clinically relevant improvements in asthma control which are disconnected from pulmonary function outcomes such as FEV_1(15, 16). FeNO correlates well with peripheral blood eosinophils (17) and total IgE in patients with partially controlled asthma(18). FeNO levels are suppressed by inhaled corticosteroid therapy (19). Levels of FeNO can measure effectiveness of corticosteroid therapy as well as compliance with treatment (20).

IL-5 promotes eosinophil recruitment, eosinophil chemotaxis to the airway and eosinophil accumulation and survival. Eosinophils release cytotoxic granules containing basic proteins such as eosinophilic cationic protein (ECP), cytokines and cysteinyl leukotrienes, causing inflammation, bronchial smooth muscle spasm, and cellular damage. Corticosteroids, the cornerstone of asthma treatment, reduce the numbers of eosinophils in blood and sputum by inhibiting the expression of pro eosinophilic cytokines such as IL-5, and increase the rate of apoptosis and associated phagocytosis. Pointedly, even low to medium doses of inhaled corticosteroid therapy in asthma can reduce both blood eosinophils and FeNO, in a dose dependent manner (17).

Whilst inhaled corticosteroids treat type-2 airway inflammation, bronchodilator drugs in the form of long acting beta-2-agonists (LABA) and long acting muscarinic receptor antagonists (LAMA) are used as add on therapies over and above corticosteroids. Their predominant function is to oppose the pro-contractile smooth muscle effects of the cascading type II airway inflammatory process, essentially improving lung function. It is uncertain to what extent LABA and LAMA possess clinically important anti-inflammatory properties, beyond their bronchodilator function. LABA in combination with ICS may possess a steroid sparing property by activating glucocorticoid receptors and enhancing corticosteroid sensitivity (21, 22), However LABA monotherapy possesses no clinically important anti-inflammatory effect (23) and LABA monotherapy is associated with increased airway inflammation from expression of IL-6, an effect which disappears with the addition of ICS (24). In vitro it has been demonstrated tiotropium (a LAMA), may have non neuronal anti-inflammatory effects, as it attenuates IL-13 induced goblet cell metaplasia, on human airway epithelium (25), a key process in type II airway inflammation. In vivo however, it has been demonstrated that the efficacy of tiotropium is independent of the Th-2 phenotype (26). Furthermore, tiotropium when added to high dose ICS/LABA in severe persistent non-smoking asthma, results in no measurable reduction in biomarkers of Type 2 inflammation, such as ECP and only marginal reduction in NO (27). When comparing add on LAMA (tiotropium) versus add on LTRA (montelukast) or doubling ICS, in asthma patients receiving ICS/LABA, superior FeNO reductions were seen in the LTRA and double dose ICS arms (28). Thus, suggesting any anti-inflammatory property it possesses in asthma may only be marginal.

Non-type 2 airway inflammation.

Smoking and air pollution can result in a non-Th-2 derived airway inflammation. The constituents of cigarette smoke or heavy air pollution damage the epithelial lining of the oral cavity and airways; a process which can be independent of or coexist with Th-2 airway inflammation.

Whilst it is known that patients with asthma have a significantly higher risk of developing COPD than those without asthma, even after adjusting for smoking history (29), unequivocally, smoking is still significantly associated with increased risk of acquiring COPD. People who smoke ≥15 cigarettes/day have an eight times higher risk of developing COPD compared to non-smokers (30). Inhaled primary and secondary cigarette smoke alongside other pollutants (such as burning biomass) result in epithelial cell and macrophage activation. Both these cells release chemotactic factors (chemokine ligands), which attract inflammatory cells to the lungs. Chemokine ligand 2 (CCL2) acts on chemokine receptor 2 (CCR2) to attract monocytes, monocytes then differentiate into further macrophages in the lung, resulting in ongoing inflammation. Chemokine ligand 1 and 8 (CXCL1, CXCL8), also released by macrophages and epithelial cells promote neutrophil attraction. Neutrophils release proteases which promote alveolar wall destruction, and mucous hypersecretion. Epithelial cells also release CXCL9, 10 and 11 which act on a receptor on T-Helper 1 (Th-1) and type 1 cytotoxic T cells (Tc-1) to promote their recruitment. These cells release proteases which result in elastin degradation and emphysema. Finally, both epithelial cells and macrophages release transforming

growth factor –β (TGF-β), which stimulates fibroblast proliferation and resultant small airway fibrosis.

The role of inhaled corticosteroids in the treatment of this non Th-2 inflammation is questionable (31), with no evidence of reduction in inflammatory cells, cytokines or proteases, in patients with COPD, even when treated with high doses of ICS (32). In a study of 60 patients with COPD given six weeks of ICS vs placebo, there were no overall treatment associated changes in forced expiratory volume in 1 second (FEV1), chronic respiratory disease questionnaire score, or sputum characteristics (33). Only when dividing the data into tertiles was an improvement in FEV_1 of 110ml noted compared with placebo in the highest tertile. Interestingly this improvement, whilst above the MCID of 100ml (34), was not associated with a fall in the sputum eosinophil count.

The add on role of inhaled corticosteroids to dual bronchodilator therapy is also marginal in terms of its effect on reducing exacerbations with one recent study demonstrating a difference of 0.09 exacerbations per year when ICS was added to LABA/LAMA (35). Therefore, aside from smoking cessation, the mainstay of pharmacological treatment for COPD is bronchodilator therapy.

There is emerging research into a clinical description of patients that have aspects of both Type 2 and non-Type 2 airway inflammation, the so called asthma-COPD overlap (ACO) (36) Controversy exists as to whether this condition truly exists or whether it compromises overlapping features between two different diseases (such as eosinophilia or reversibility), the term ACO has been removed from the more

recent GOLD COPD statement (37), but is still referred to in current scientific literature (38). This may be because, in certain patients, differentiating asthma and COPD at a diagnostic level is often challenging. ACO may be considered a broad categorisation of patients who have features of both diseases, but is not clearly defined, as ACO does not represent a single phenotype. It can include patients with COPD and eosinophilic inflammation, patients with severe asthma and irreversible airway obstruction, and patients with asthma who smoke and have non-Type 2 airway inflammation. Hence, to label ACO as a syndrome, may be misleading (6). There may however be a clinically relevant subgroup of COPD patients who have Type 2 (asthma-like) gene expression alterations. If such patients could be adequately characterised with a biomarker (such as eosinophil count), it would enable delineation of those who would benefit from Type 2 targeted therapies such as ICS.(39), versus bronchodilation alone.

Therefore, in terms of the role of inhaled pharmacotherapy, perhaps asthma and COPD comprise a spectrum of disease that must be treated with *targeted* therapy (40). Hence, in order to deliver the right treatment to the right lungs, rather than “more treatment to more lungs” (3), one must consider the mechanisms of airway disease in the spectrum of asthma and COPD, and demonstrate the role of current pharmacotherapy in managing the treatable traits of the disease. This is the hypothesis tested within this thesis.

Airway mechanics:

Airway smooth muscle tone is a principal determinant of airway diameter and resistance. In physiological conditions, airway smooth muscle has little tone, the

airways are patent and airway resistance does not limit breathing (41). In obstructive airway diseases such as COPD and asthma, there is increased airway smooth muscle tone. This is due to the production of multiple agents that promote airway smooth muscle contraction, via G-protein coupled receptors. The two most important receptors contributing to increased airway smooth muscle tone are the M3 muscarinic acetylcholine receptor (mAChR) , activated by acetylcholine released by the nerves of the parasympathetic nervous system; and the beta-2-adrenoceptor (B2AR) G-protein coupled receptor, which antagonises airway smooth muscle contraction.

Whilst airway smooth muscle tone is principally regulated by the M3 receptor, most muscarinic receptors on airway smooth muscle are M2, which contribute indirectly to airway tone. M2 in the airway smooth muscle is responsible for limiting B2AR mediated relaxation. M2 receptors present on parasympathetic nerves supplying the lung limit acetylcholine release (42), a so-called cholinergic brake, providing negative feedback control over acetylcholine release. In order to provide effective bronchodilation, a muscarinic receptor antagonist should inhibit M3, but not M2, to spare potentiation of vagally induced bronchoconstriction (43).

Receptor crosstalk refers to instances in which components of one signal transduction pathway affect another (44). Crosstalk exists between the B2AR and the mAChR (41). Beta-2-agonism results in M3 receptor desensitisation, via protein kinase A. This results in functional antagonism of pro-contractile signalling. Crosstalk in the other way also exists, with M3 mAChR activation promoting B2AR

desensitisation, via protein kinase C; this results in a diminished capacity to relax muscle.

Role of long acting bronchodilators in airway disease:

In asthma, long acting beta agonists and muscarinic receptor antagonists are given as add on therapy to inhaled corticosteroids (ICS) (45). Long acting beta agonists are always given in combination with inhaled corticosteroids to mitigate the known problem of B2AR tachyphylaxis and desensitisation (46) (47) (48, 49). Prolonged occupancy of the B2AR by an agonist (formoterol, salmeterol etc.), resulting in rapid uncoupling and internalisation of the receptor from cell surface. Both inhaled and oral glucocorticoids restore B2AR density on the surface of cells that have undergone B2AR downregulation in the presence of an agonist (50-54). Clinically this is borne out by large RCTs; Lazarus *et al*. demonstrated that in patients with persistent asthma, those prescribed salmeterol monotherapy, versus those prescribed low dose ICS (triamcinolone) monotherapy had significantly increased asthma exacerbations (20% vs 7% p=0.04), significantly higher treatment failures (24% vs 6% p=0.004), and equivalent peak expiratory flows, and rescue salbutamol use (55). In a Cochrane review of seventy seven studies where LABA was added on to inhaled corticosteroids versus same dose inhaled corticosteroids, in adults the addition of a LABA at licensed doses reduces the rate of exacerbations requiring oral steroids, improves lung function and symptoms and modestly decreases use of rescue short-acting beta-2-agonists (56). In a post marketing US FDA-mandated safety study, in 11,693 adolescents and adults with moderate-severe asthma, treatment with ICS/LABA (budesonide/formoterol) versus ICS alone, was associated

with a lower risk of asthma exacerbations (hazard ratio, 0.84; 95% CI, 0.74 to 0.94; P=0.002), and an equivalent risk of serious asthma related events (57). These data, therefore, confirm current British Thoracic Society guideline that LABAs are the first choice add on therapy to ICS.

LAMAs are currently positioned as add on to ICS/LABA in asthma (45), currently tiotropium is the only licensed add on LAMA. Tiotropium (TIO) is a long acting muscarinic antagonist (LAMA), which is functionally selective for the postjunctional M3 muscarinic receptor, found on airway smooth muscle (43), this “functional selectivity” is due to the ability of tiotropium to dissociate from the M2 receptor ten time faster than the M3 receptor (58). TIO reduces asthma exacerbations by 21% in patients when used as add on therapy in patients receiving inhaled corticosteroids and long-acting beta-agonists (ICS/LABA)(59). Whilst blocking the M3 receptor inhibits acetylcholine-induced bronchoconstriction, TIO exhibits only modest improvements in forced expiratory volume (FEV_1), which amounts to approximately 100ml at trough (59, 60), which is less than the minimally important difference of 230ml (61). It is therefore hard to explain the protective effect on exacerbations on the basis of this small improvement in airway calibre alone (62). Neither can the reduction in exacerbations afforded by add-on tiotropium be correlated with improvements in the asthma quality of life questionnaire (AQLQ) (63). Asthma severity, judged by the AQLQ, significantly relates to future risk of asthma exacerbation, over and above the risk conferred by prior exacerbations (64). In a severe asthma study, when tiotropium vs placebo was added to ICS/LABA for 4 weeks, tiotropium failed to show improvement in AQLQ (27, 59).

Therefore, the proposed mechanism through which tiotropium reduces exacerbations remains unclear. One possible mechanism may be by conferring a bronchoprotective effect against exogenous constrictor stimuli (challenge). The sensitivity of the airways to constrict upon exposure to agonists (challenge) is termed airway hyperresponsiveness (AHR). In murine models of acute and chronic asthma, treatment with tiotropium bromide significantly reduced airway inflammation, AHR, and Th2 cytokine production in bronchoalveolar lavage fluid (BALF) (65). In a Guinea pig model of asthma administration of tiotropium resulted in complete blockage of antigen-induced AHR (66). Hence these animal data suggest a potential anti-inflammatory and bronchoprotective action of tiotropium, which requires further evaluation in humans.

Airway hyperresponsiveness

AHR is a hallmark feature of persistent asthma and can be regarded as a surrogate marker of type II airway inflammation. AHR is often present in well controlled asthma (67), It is linked to airway remodelling even in asymptomatic subjects (68). Severity of AHR relates to levels of type II inflammatory biomarkers (69), as subjects with AHR have significantly higher levels of sputum eosinophils, ECP, and peripheral blood eosinophils, compared to those without demonstrable AHR (69, 70). In mild asthma the level of AHR also correlates to sputum eosinophils (71). AHR is known to relate disease severity (72) ,with more severe AHR being associated with steeper falls in FEV_1 over time and increased symptoms (73).

To assess airway hyperresponsiveness patients inhale “challenge” agents which can be via a dry powder inhaler or via a nebulised dosimeter, the procedure is termed a bronchial challenge (or bronchoprovocation) test. The severity of AHR is measured by calculating the provocative concentration (PC), or provocative dose (PD) of challenge agent, required to reduce FEV_1 by 20% (PC20 or PD20 respectively). Pulmonary function (traditionally FEV_1) is measured at the start of the procedure, prior to any challenge being administered, and then after each dosing step. A pre-specified cut off such as a drop in FEV_1 by 15 or 20% is determined a positive challenge. In principle, bronchoconstriction to the lowest doses of challenge agent suggest marked airway hyperresponsiveness, whereas lack of bronchoconstriction to the highest doses of challenge agent suggest little to no AHR. A total lack of AHR to a standard challenge test may point to a diagnosis other than asthma (74).

Many patients with COPD also have evidence of AHR: In the Lung Health Study, two thirds of the approximately six thousand participants with mild or early COPD, had demonstrable AHR (75). The severity of AHR predicted subsequent decline in lung function in COPD. The utility of assessing AHR in COPD is unclear as interpretation of AHR is hampered by how dependent it is on baseline FEV_1 (69). In severe COPD therefore it may be argued that AHR in COPD is merely a surrogate marker of airflow obstruction, additionally, the test is generally contraindicated in those with an FEV_1 of less than sixty percent predicted (76). It should be noted that the dependency of degree of AHR on baseline FEV_1 is numerically the same in asthma and COPD (77). It is known that short term treatment with high dose budesonide does not improve hyperresponsiveness to indirect bronchial challenge in patients with

(mild/moderate/severe) COPD (78). Moreover even prolonged treatment with ICS for two years has no impact on AHR in patients with (mild/moderate/severe) COPD (79), again suggesting that the role of ICS in treating AHR in patients with (mild/moderate/severe) COPD is limited. Little work has been done however, on the role of bronchial challenge and interaction on bronchodilator therapy in COPD.

Bronchial challenge testing can be completed using direct or indirect acting bronchoconstrictor agents (stimuli), referring to the mechanism by which a stimulus mediates bronchoconstriction (figure 1).

Methacholine or histamine are examples of direct bronchial challenge agents that cause bronchoconstriction by specific receptor action on muscarinic or histamine receptors situated on airway smooth muscle, and hence a fall FEV_1. Direct challenges are more sensitive than indirect challenges, however they are less specific: Positive challenges may occur in many individuals with no asthma symptoms (80), however negative direct airway challenges can be used to exclude current asthma in a clinic population (81).

Naturally occurring stimuli such as viruses, allergens, chemicals, exercise and cold air cause bronchoconstriction through indirect mechanisms, acting on inflammatory and neuronal cells which release mediators or cytokines to cause downstream bronchoconstriction (82). Hence the more physiological nature of an indirect airway challenge is suggested to have more clinical relevance (80, 83), and is more specific in the diagnosis of asthma, with one population study demonstrating a specificity of 98.4% (95%CI, 96.2% to 99.4%) (81, 84). Moreover, it has been suggested that

indirect airway challenge should correlate better with asthma activity (85) and level of eosinophilic airway inflammation, compared to direct (86) and show greater improvement with both allergen avoidance compared to direct (87). Examples of indirect stimuli include adenosine 5' monophosphate and mannitol. Mannitol is thought to more closely reflect physiological stimuli and acts by the releasing pro-inflammatory mediators (88). Moreover mannitol challenge has been shown to be related to an TH2 phenotype in asthma (89-91).

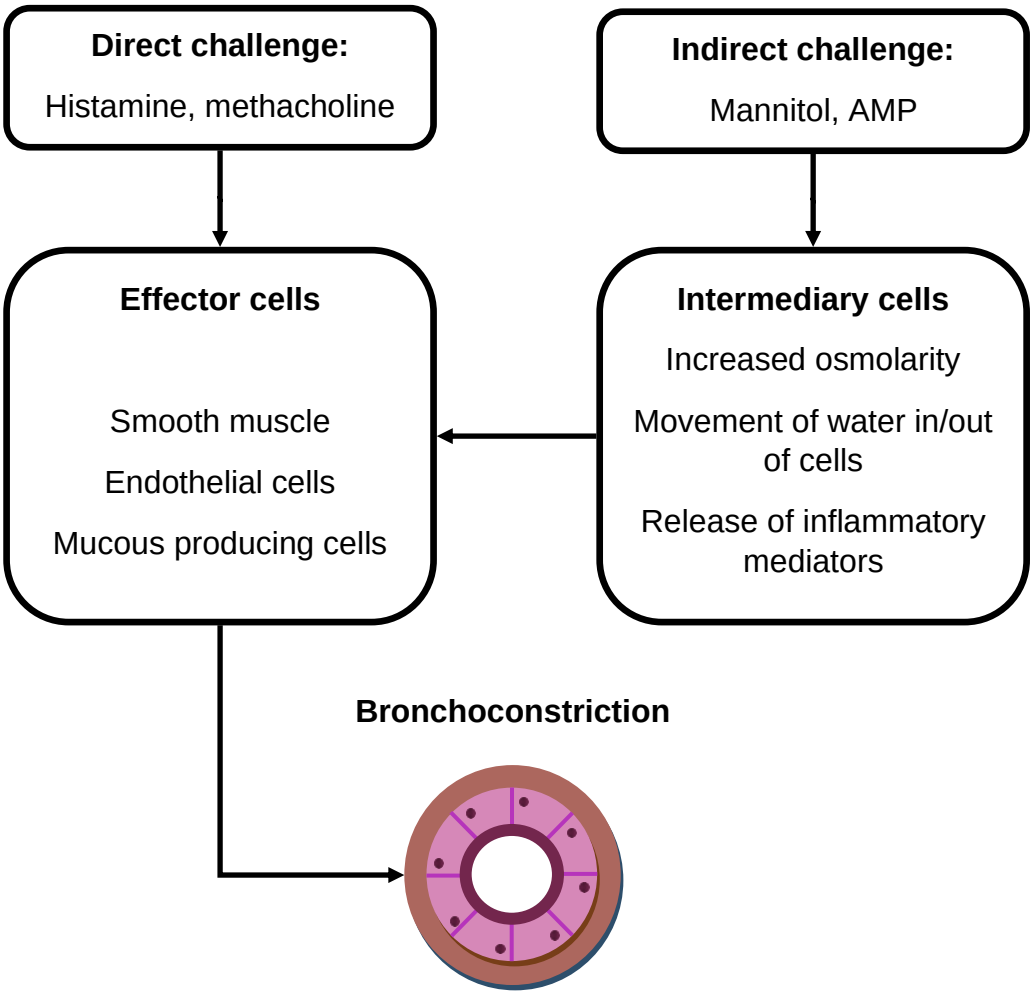


Figure 1

Summary of direct vs indirect challenge.

Traditionally, the pharmacological management of asthma has been based solely on symptoms and conventional lung function, rather than AHR or measures of inflammation, either direct or surrogate. Titrating anti-inflammatory ICS therapy to the level of AHR as measured by indirect BCT has previously been shown to reduce exacerbations and improve symptoms compared to a non-AHR driven strategy (92). Measuring AHR is a useful non-invasive method to confirm or refute a diagnosis of asthma when lung function is preserved, and symptoms are equivocal. It may also be used to adjust the level of anti-inflammatory treatment - in a study by Sont et al,. patients whose therapy was targeted solely against AHR exhibited fewer exacerbations , higher FEV1 and less airway remodelling (93).

Smoking in asthma and COPD:

It is well established that tobacco smoking enhances airway hyperresponsiveness in patients with and without an overt diagnosis of asthma (94-96). Smoking cessation results in reduction in AHR to indirect challenge, but not direct challenge, in as little as six months post cessation (97) At twelve months post cessation both indirect and direct challenges show similar improvement. This would suggest that indirect challenge agents detect the presence of reversible inflammation induced by tobacco smoke earlier than direct agents.

Increased airway resistance is common in smokers, even when conventional pulmonary function (FEV_1) is preserved (98). This may suggest that early small airway damage is occurring, which may eventually lead to COPD. As IOS can demonstrate small airway dysfunction and relates closely to asthma control (99, 100)

it may be a useful tool to assess this.

Compared to non-smoking asthma, those with asthma who smoke have significantly poorer asthma control, independent of FEV_1 (101). Furthermore, cigarette smoking in asthma is associated with a higher frequency of exacerbations, increased number of life-threatening asthma attacks and asthma mortality is greater among heavy smokers with asthma compared to never smoking asthma (102, 103). This has a huge impact on health care resources due to unscheduled doctor visits and frequent hospital admissions. Despite this, smoking cessation rates are very low due to the highly addictive nature of tobacco smoking and the prevalence rate of smoking among asthmatics is similar to that of the general population (104). Patients with asthma who smoke are particularly challenging to manage because they are resistant to the beneficial effects of corticosteroids, the mainstay of asthma treatment (105, 106). Furthermore, they have substantially greater declines in FEV_1 over time, than non-smokers (107).

Unfortunately, there is no guideline consensus regarding how to best manage smoking asthma patients. Therapeutic studies in asthma tend to exclude smokers because of concerns about recruiting patients with chronic obstructive pulmonary disease (COPD) (108); two recent studies looking specifically at the efficacy of tiotropium in asthma excluded any current smokers or those who had a pack year history of greater than 10 (109, 110). Hence, there is an unmet need for therapeutic studies in people with asthma who continue to smoke.

Compared to non-smokers, smokers have greater cholinergic airway tone, patients with COPD have a greater still tone (111). When administered the anticholinergic agent atropine, non-smokers had the smallest increase in FEV1, compared to smokers, then those with COPD. When smokers with asthma and non-smokers with asthma are administered ipratropium, a short acting muscarinic receptor antagonist, they have a greater bronchodilation effect compared to non-smokers (112). Additionally, in the same study, when ipratropium and salbutamol were given together smokers had significantly greater synergistic bronchodilation effect compared to non-smoking asthma, receptor crosstalk may be a plausible explanation for this effect. In a small single dosing study of tiotropium added to ICS/LABA in smoking vs non-smoking asthma, those with higher pack-years or lower baseline percentage FEV_1 showed greater increases in FEV1 in response to tiotropium (113). The limitations of this study were its single dosing, hence the potential for this effect to disappear after chronic dosing remains.

Tiotropium, when added to ICS/LABA, may reduce airway resistance as well as attenuate AHR. It is uncertain whether this relates to an anti-inflammatory effect in smokers. Cigarette smoke induces interleukin-8 release from human bronchial epithelial cells (114); it has been suggested that this increases airway inflammation. Costa *et al* have shown that combining the ultra-long acting $beta_2$ agonist olodaterol with tiotropium gives synergistic benefit in terms of anti-inflammatory response, with statistically significant reductions in IL-6 and IL-8 levels (115) observed in vitro with human lung fibroblasts. Olodaterol and tiotropium also significantly restored cAMP levels in fibroblasts of asthma subjects, beyond the levels induced by the agents

individually, suggesting synergism. This cAMP-dependent signalling pathway provides negative feedback for inflammatory responses. Whether this correlates to a measurable benefit in lung function remains to be seen.

Tobacco use predisposes those with asthma to develop chronic obstructive pulmonary disease (COPD). It is also associated with significant cardiovascular comorbidity (116), independent of degree of airflow limitation; whilst severity of airflow limitation does significantly relate to breathlessness, health status, walk distance and exacerbations (117). Large population data has demonstrated that the mortality of patients with COPD increases with severity of AHR, even when adjusted for sex, age, smoking, and lung function amongst other variables (118), yet little emphasis is placed on AHR in guideline based management of the disease. This is despite a traditional fear of prescribing drugs which theoretically could induce AHR, such as beta-2-receptor antagonists in COPD (119).

The severity of airflow limitation in COPD is based on FEV_1 which strongly correlates with burden of small airway disease, even before the onset of emphysematous destruction (120). Small airway disease has been shown to be associated with frequent COPD exacerbations (121)

The initiation of inhaled COPD therapy depends on disease staging, as guided by management strategies, such as GOLD (7). This assesses patients based on symptoms and exacerbations and categorises them into four risk groups from A to D. Groups A and B are patients who experience <2 exacerbations per year requiring steroids/antibiotics, and no hospitalisations. Groups C and D are high risk patients,

who are experiencing ≥2 exacerbations per year (requiring steroids/antibiotics), or one hospitalisation. Stratification between A to B, or C to D is symptom based, assessed by the modified Medical Research Council (mMRC) dyspnoea score, or the COPD Assessment Test (CAT) score.

Long acting bronchodilator therapy is the preferred therapy in GOLD A-C patients. In exacerbation-prone patients with high symptoms (GOLD D) (7), triple inhaled therapy (ICS/LABA/LAMA) is recommended. Tiotropium, when added to ICS/LABA has been shown to significantly reduce all-cause mortality, hospital admissions, and oral corticosteroid bursts in patients with COPD, when compared to ICS/LABA alone (122). Moreover, tiotropium, in particular, has been shown in a large (n=5993), four-year, randomised double blind trial, to reduce mean number of exacerbations by 14% (P<0.001), and time to first exacerbation, (16.7 months, 95% CI, 14.9 to 17.9), compared to usual inhaled therapy (12.5 months, 95% CI, 11.5 to 13.8) (123).

Small airway disease and its assessment with impulse oscillometry:

The small airways (<2mm diameter) account for 98.8% of the total lung volume, and are located from the eighth generation of the airway and below (124). The small airways are implicated as being the predominant site of airflow obstruction, irrespective of the whether the pathogenesis of the airway disease is due to asthma or COPD. (125). The small airways are often referred to as the “quiet zone” (126), as extensive airway disease can be present with only mild abnormalities in conventional pulmonary function.

Small airway disease (SAD) is evident in over 50% of moderate to severe asthma with preserved (>80% predicted) FEV_1 (127). Moreover, persistent small airway dysfunction in asthma is associated with significantly higher long term oral corticosteroid and salbutamol use, despite preservation of FEV_1 (>80% predicted)(128). Small airway disease is also a key feature of COPD, and correlates directly with disease severity (120). It is present in the early stages of the disease, prior to onset of emphysema. Progression of COPD is strongly associated with increases in tissue volume in the wall of the small airways and accumulation of mucous in their lumens (129).

The assessment of small airway disease in asthma and COPD is challenging because the region is relatively inaccessible for functional measurements. Forced expiration manoeuvres such as conventional spirometry to measure FEV_1 are not specific to SAD and are non-physiological and carry the key disadvantage of being effort dependent. Impulse oscillometry (IOS) is a pulmonary function test which can be used to assess small airway disease and response to bronchodilator therapy (130).

IOS is an effort independent method of measuring airway resistance (R) and its reciprocal reactance (X). It is a form of forced oscillation technique, first described by Dubious *et al*. in 1956 (131). Impulse oscillometry transmits square wave pressure at a fixed frequency (usually 5Hz), from which all other frequencies of interest are derived. The pressure oscillations conduct along the bronchial tree causing distension and recoil of the lung parenchyma. Pulmonary resistance

measures the energy required to propagate the pressure wave through the airways. High frequency waves ≥20 Hz travel short distance and typically measure resistance of the proximal airways, therefore, the resistance at 20Hz (R20) represents proximal airway resistance. Lower frequency waves travel further into the lung parenchyma and reach out to the small airways; hence the resistance at 5Hz (R5) represents the total airway resistance. As disease in the peripheral airways will increase total airway resistance (R5) to a greater extent than proximal airway resistance (R20), this is known as a frequency dependent change, or heterogeneity of resistance. Therefore, by subtracting the value of central airway resistance from the total airway resistance (R5-R20), the frequency-dependent heterogeneity (i.e. degree of obstruction) of the small airways can be measured. Reactance (X) is a measure of the energy generated by the recoil of the lung after distension by the pressure wave. The area of reactance (AX) is an area under the curve between reactance values for 5Hz and the resonant frequency (Fres, Hz), the frequency at which reactance is zero.

IOS is particularly useful in patient groups who may struggle to adequately perform effort dependent airway manoeuvres, such as children, those with physical limitations, and the elderly. It gives a better insight into lung mechanics compared to spirometry, which only measures volume and flow. Whilst it is acknowledged that body plethysmography may be considered an alternative method to assess airway resistance (sRaw) and conductance (sGaw), it is a technically demanding assessment (132). The complex panting manoeuvres required (for up to five breathing loops), can be exhausting particularly for patients with impaired lung function. Moreover Raw reflects resistance of the bronchial tree, whereas

oscillometry measurements reflect total respiratory system resistance (133) and can be used to extrapolate small airway resistance (R5-R20). Pointedly, R5-R20 and AX are more sensitive than sRaw and sGaw in terms of detecting changes in airway geometry post indirect (allergen) and direct (methacholine) challenge (134).

As small airways dysfunction is highly predictive of worse asthma control (100) and relates to COPD disease severity (135), it is conceivable that assessment of this as an outcome may pick up early improvements of bronchodilator pharmacotherapy, which are not detected using FEV1. IOS has been used in all studies within this thesis to assess degree of small airway dysfunction alongside conventional spirometry.

Hypothesis:

Study 1: Non-smoking asthma (NCT02039011): This study chose to assess the bronchoprotective role of ICS/LABA/LAMA versus ICS/LABA on patients with non-smoking asthma. These patients were challenged with the indirect challenge agent mannitol using conventional cut offs for positive challenge i.e. the provocative dose required to reduce FEV_1 by a given percentage. The hypothesis was that addition of LAMA would confer superior bronchoprotection to mannitol than ICS/LABA.

Study 2: Smoking asthma (NCT02039011): This study assessed the bronchoprotective role of ICS/LABA/LAMA versus ICS/LABA on patients with asthma who smoke. We again utilised the challenge agent mannitol, but on this occasion had a more exploratory primary outcome. We hoped that there would be a

greater sensitivity in terms of signal to noise with an IOS outcome measure compared to conventional FEV_1. Therefore, we chose to look at the provocative dose required to increase total lung resistance (R5) by a given percentage. It was hoped that this would highlight the utility of impulse oscillometry in the assessment of airway disease.

Study 3: Beta blockers in moderate to severe COPD (NCT01656005): Conventional airway challenge is prohibited in patients with an FEV_1 of <60%. We chose to include a real-world COPD population (moderate to severe) and so aimed to recruit those with an FEV_1 even as low as 30% predicted. Therefore, it would be unethical to expose them to significant and sudden bronchoconstriction. However, we still wished to assess the bronchoprotective role of ICS/LABA/LAMA vs ICS/LABA vs ICS. To explore the potential benefit bronchodilators may have conferred in these patients selective (bisoprolol 5mg OD) and non-selective (carvedilol 12.5mg BD) beta blockers were given to the patients in therapeutic doses. Once at a steady state the bronchodilators were sequentially withdrawn (LAMA then LABA). This allowed the beneficial effect of each to be isolated. Furthermore, as lower prescribing rates for beta-blockers have been described in COPD patients with heart failure or post-myocardial infarction (119, 136-138), this study also aimed to address concerns over pulmonary tolerability of these drugs.

The commonality between all three studies was the LAMA (tiotropium) which was the only LAMA licensed at the time across asthma and COPD.

Methods:

The following randomised controlled trials were included in this thesis:

- Proof of concept study to evaluate single and chronic dosing effects of ultra-long acting bronchodilator therapy on mannitol challenge in asthma patients taking inhaled corticosteroids.
- Effects of ultra-long acting bronchodilator therapy assessed by impulse oscillometry in smoking asthma taking inhaled corticosteroids
- Beta-Blocker Therapy in Moderate to Severe COPD

Non-study specific methodology:

Participant selection:

Patients were selected from a database of existing volunteers at the Scottish Centre of Respiratory Research (SCRR), at the University of Dundee, and from primary and secondary care, within the health boards of NHS Tayside and Fife. Participants were also recruited through the Scottish Primary Care Research Network (SPCRN). Patients agreed to be contacted for current and future studies, and details were kept securely in a locked office. Patients were invited to attend a general screen for either asthma or COPD, and thereafter offered participant information leaflets on recruiting studies suited to them. After a minimum of 24 hours patients were contacted to enquire whether they wished to participate in a trial, if in agreement they were

booked for a study specific screening visit. All participants provided full written informed consent and were a minimum of 18 years of age at the time of enrolment into a study. In order to enter an asthma or COPD study, patients had to have a physician diagnosis of the respective disease, have a physical examination, and no significant comorbidity which could jeopardise their safety or protocol integrity. Specific entry criteria are provided within each chapter for the individual studies. The Scottish Primary Care Research Network was also utilised to enhance recruitment from primary care. All study staff maintained up-to-date good clinical practice (GCP) training, all study documents were approved by the Sponsor, East of Scotland Regional Ethics Committee (REC 2), NHS Tayside Research & Development and MHRA, prior to study commencement.

Overarching inclusion/exclusion criteria:

Inclusion: Agreement for their GP to be made aware of study participation and to receive feedback as relevant to the participant's wellbeing.

Exclusion: Participation in another trial within 30 days before the commencement of the study; pregnancy or lactation; unable to comply with the procedures of the protocol; any clinically significant medical condition that may endanger the health or safety of the participant; inability to give informed consent. Known or suspected sensitivity to/intolerance of IMP. An asthma, COPD exacerbation, or respiratory tract infection requiring systemic steroids and/or antibiotics within 1 month of the study commencement or 3 months if hospital admission was required.

Principles regarding data and statistical method:

Whilst each study has a specific description of the statistical methods used and power calculations within its respective section, the following principles applied to all data and its management:

Data from study visits was entered into a participant paper case report form (CRF) at the time of measurement. Data collected from CRFs was entered into EXCEL following Tayside Medical Science Centre standard operating protocols. All data was assessed for normality, distribution, and outliers. When required, data was log transformed if non-normally distributed. Due to the small datasets, all analysis was per protocol only. All studies recruited enough patients to meet pre-specified powering. Statistics were completed solely by the PI with oversight from the CI.

All statistics were carried out using IBM SPSS V22 (IBM Analytics, New York, USA). Graphing was completed using GraphPad Prism (V 6.0, California USA). For all studies, statistical significance was defined as an alpha error of 5% (two tailed), with 95% confidence intervals given for mean changes where appropriate.

Randomisation was done to Good Clinical Practice (GCP) standards, with a computer-generated code held by the Clinical Trials Pharmacy, Ninewells Hospital, Dundee.

Trial Management:

All trials were co-ordinated by a Trial Management Group TMG), consisting of the Chief Investigator, Principal Investigator and Research Group Facilitator. The Research Group Facilitator was responsible for checking the CRFs for

completeness, plausibility, and consistency. The Principal Investigator had oversight of all studies and was accountable to the Chief Investigator. A data monitoring committee was not required as there was oversight via the Trial Management Group. The University of Dundee and Tayside Health Board co-sponsored all studies. The end of the study was defined as the final visit of the final participant. No study drug was continued following the end of the study. Participants were returned to their usual medication after the study.

Measurements:

- Spirometry:

  Spirometry was carried out in accordance with guidelines published by the American Thoracic Society (ATS) (139), using a SuperSpiro (Micro Medical; Rochester, Kent, UK) spirometer. Patients were asked to sit upright, exhale, inhale rapidly and completely, position themselves on the mouthpiece, and then exhale with maximum force until completion. This was done until there were three readings, of which the best two were within 100mls or 5% of each other. Forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC), FEV_1/FVC ratio, and forced mid expiratory fraction (FEF_{25-75}) were recorded; alongside percentage predicted results for normal population distributions for the patient's respective age sex, height, and ethnicity. Prior to spirometry short acting reliever therapies such as salbutamol and ipratropium were withheld for at least 6 hours. Each study had specific

withholding times per protocol and these are detailed in their respective method sections.

- Impulse oscillometry (IOS):

  IOS, described in detail above, was carried out using MasterScreen IOS, Viasys Healthcare, Leibnizstrasse 7, D-97204 Hoechberg, Germany, to measure airway resistance (R) and reactance (X) in accordance with the manufacturer's instructions. The main parameters in IOS are: R5, R20, R5-R20, X5, AX, Fres. The IOS instrument is calibrated daily using a 3L syringe for resistance. Patients are seated in front of the machine, with legs uncrossed, and nose clip worn. They then place their mouth on the mouthpiece with a tight seal, to prevent air leak. The cheeks should be held firmly by the patient (or assistant), thereafter they perform normal tidal breathing for at least 30 seconds. During this period 120-150 sound impulses are pushed into the lungs via a loudspeaker. A pressure and flow transducer measures inspiratory and expiratory pressure and flow of tidal breathing, and that of the superimposed oscillation signals. A signal filter separates returning signals from breathing pattern. From this mean reactance and resistance are determined at frequencies from 5 to 20 Hz (140). To have reproducible results, a minimum of three tests are performed. Tests with significant (cough etc.) artefacts are discarded. Quality assurance is measured by coherence, a value between zero and one which reflects reproducibility of measurements, - this should be between 0.8 and 1.0 (140).

- Bronchial challenge testing: Assessing airway hyperresponsiveness

  Within this work mannitol (Osmohale, Pharmaxis, Sydney, Australia) was the chosen challenge agent. This was carried out as previously recommended (141). Prior to the challenge, spirometry should be performed and baseline FEV1 established.

  The patient applies a nose clip and breathes through their mouth. The following cumulative doses steps are administered through a dry powder inhaler device with dry powder capsules inserted within it: 0mg, 5mg, 15mg, 35mg, 75mg, 155mg, 315mg, 475mg, 635mg. 60 seconds after each completed dose FEV1 is measured. A positive response per manufacturer's guidelines is achieved when the patient experiences a 15% fall in FEV1 from baseline (0 mg dose).

  Mannitol sensitivity is expressed as the provocative dose of mannitol required to reach a 15% drop in FEV_1 (PD_{15}) This was calculated by interpolation of the log-linear dose–response curve. The data for PD15 were log transformed before analysis Mannitol reactivity is expressed as the response dose ratio (RDR: max % fall in FEV1 / cumulative dose).

- Fractional exhaled nitric oxide (FeNO):

  FeNO was carried out using NIOX MINO or VERO (Aerocrine AB, Solna, Sweden) according to manufacturer's instructions. The VERO and the MINO have acceptable agreeability and are highly correlated (142). FeNO is

measured by inhaling to total lung capacity, followed by exhalation into a mouthpiece at a steady flow rate for 6-10 seconds, with just one successful effort required to complete the test (143). It is measured in parts per billion (ppb). Cigarette smoking suppresses FeNO by altering lung mechanics and therefore was not measured in the study involving current smokers (144).

- Skin prick test:

  This was performed according to standard protocol using standardized allergen extracts (Diagenics Ltd, Milton Keynes, UK) including house dust mite, cat, dog, grass, tree, weed pollen, feather, and aspergillus, in addition to a negative and positive control. One drop of each allergen was placed on the volar aspect of the forearm, and a prick lancet is pushed through the drop allowing a very small quantity of the solution to penetrate the epidermis. A positive reaction is indicated by the wheal being 2mm greater than the negative control, after approximately 15 minutes. Skin prick testing is more sensitive than radioallergosorbent testing for aeroallergens (145). Antihistamines are withheld 5 days prior to skin prick testing.

- Quality of life & functional assessments:
  - Diary cards: Participants performed and recorded twice daily domiciliary pulmonary function measurements (either PEF or FEV_1) completed a daily diary.of study inhaler use, reliever use and symptoms on global 0-3 scale (0=none, 1=mild, 2=moderate,

3=severe), from the beginning of the step-down/run-in period through to the end of the study. Patients were given a reference peak flow value (70% of their best reading from screening), which defined a safety threshold. A peak flow reading below this required them to contact the study group and be assessed by medical staff. Patients were trained how to use a peak flow meter (Mini-Wright standard range), or Piko-6 digital lung function meter (Nspire Health Inc.) for FEV_1, to record their domiciliary lung function. The purpose of the diary card was to assess compliance with inhaled therapy and for safety purposes also. A minimum of 80% compliance with the investigational medicinal product (IMP) was expected during the study.

- Asthma control questionnaire (ACQ): This is a seven domain questionnaire (Qoltech, UK) used to assess overall asthma control (146), each domain is worth a maximum of six points. The patient reports symptoms over the past week over six domains: wakening due to asthma; morning asthma symptoms; activity limitation due to asthma; asthma related shortness of breath; wheeze; and short-acting bronchodilator use. The seventh domain FEV_1 (absolute and percentage predicted) is reported by clinical staff. A score of ≤0.75 is deemed to be well controlled, and a score of ≥1.5 is not well controlled (147).
- St George's Respiratory Questionnaire (SGRQ): This is a 50-item questionnaire developed to measure health status (quality of life) in

patients with diseases of airways obstruction (148). Scores are calculated for three domains: Symptoms, Activity, and Impacts (Psycho-social) as well as a total score.

- Baseline and transition dyspnoea index (BDI-TDI): BDI measures the severity of dyspnoea at the baseline of a study. TDI measures changes from this baseline (transition period) at subsequent visits. It assess functional impairment, magnitude of task, and magnitude of effort, which provokes breathlessness (149).

- 6-minute walk test (6MWT):

  This was carried out in accordance with ATS guidelines (150). It was performed indoors on a flat, straight corridor, with a hard surface. The walking course was 30m in length, with a turnaround point marked with a cone. Patients were asked to wear comfortable clothing and shoes and use any usual walking aids during the test. The test was performed at the same time of day (mornings), and the patient was rested in a chair for at least 10 minutes before the test. During this time, vital signs (heart rate, blood pressure, oxygen saturations) were recorded. After this the patient was positioned on the start line and as soon as they started to walk a timer was started. The patient was observed, and after every minute, they were told “you are doing well, you have *x* minutes to go”. If a patient stopped during the test they were told “You can lean against the wall if you would like; then continue walking whenever you feel able”. If they stopped prematurely and were unable to go

on, the reason for stopping was recorded. If they completed the test, as the timer stopped (at six minutes), they were told to stop, and remain in position until their distance was marked with a cone. They could then rest, whilst the distance travelled was calculated. Post-test Borg dyspnoea and fatigue levels were assessed, along with a repeat of the earlier vital signs. The Borg scale (151) is a 0-10 dyspnoea and fatigue scale, 0 being “nothing at all” and 10 being “very, very severe”. Patients are asked: “Please grade your level of shortness of breath using this scale.”, then asked: “Please grade your level of fatigue using this scale.”

Study specific methodology:

Proof of concept study to evaluate single and chronic dosing effects of ultra-long acting bronchodilator therapy on mannitol challenge in asthma patients taking inhaled corticosteroids.

EudraCT No. 2013-001953-28

Research Ethics Committee (REC) No. 13/ES/0072

Clinicaltrials.gov No. NCT02039011

Inclusion Criteria: Male or female aged at least 18 years, with persistent asthma and receiving inhaled corticosteroids (at least 400 micrograms of BDP or the equivalent

daily); Participants had to have a minimum FEV1 of >50% predicted and be mannitol responsive i.e. provocative dose required to reduce FEV1 by 15% (PD15) <635mg.

Exclusion Criteria: Other respiratory diseases such as COPD, bronchiectasis or ABPA; an asthma exacerbation or respiratory tract infection requiring systemic steroids and/or antibiotics within 3 months of the study commencement; smoking within one year or >10 pack year history.

Primary outcome: Change in mannitol PD15 between single and chronic dosing of ultra-long acting bronchodilator therapy.

Secondary outcomes: IOS variables (R5, R5-R20, and AX), spirometry (FEV_1, FEV1 % predicted, FEF_{25-75}); mannitol response dose ratio (RDR), recovery in spirometry post challenge; domiciliary peak flow- (PEF); asthma control questionnaire (ACQ); post challenge recovery of FEV_1 after 400µg salbutamol; ACQ (7 point questionnaire) after chronic dosing; and FeNO.

Power: The study was powered at 80% to detect a one doubling dose in mannitol PD 15 (the primary outcome), as change from baseline, comparing indacaterol alone with indacaterol plus tiotropium, after single and chronic dosing, and a within-subject SD of 1.3 doubling dose, requiring a sample size of 14 using a crossover design, with alpha error of 0.05 (2 tailed). All data were first examined for normality and distribution. Repeated measures analysis of variance (ANOVA) was carried out assessing for treatment and sequence effects for the cross-over design. Where overall significance was found on ANOVA, Bonferroni corrected multiple pairwise comparisons were then carried out. This was necessary when comparing data within

our studies, as they featured four to six visits depending on study. Thus, pairwise comparisons are reported as either significant ($p<0.05$, two-tailed) or not. Statistical Analysis was done using IBM SPSS (version 22, IBM analytics, New York).

Beta-Blocker Therapy in Moderate to Severe COPD.

EudraCT No. 2011-006008-11

REC No. 12/ES/0054

Clinicaltrials.gov No. NCT01656005.

Inclusion Criteria: Male or female, aged 40-80 years with stable moderate to severe COPD (GOLD stage 2&3). FEV1 30-80% predicted and FEV1/FVC ratio <70%. Stable defined as no exacerbation in previous 3 months. Smoking history ≥10 pack-years. Oxygen saturations≥ 92% on room air at rest. ECG demonstrating sinus rhythm.

Exclusion Criteria: Use of domiciliary oxygen. History of other primary obstructive lung disease including asthma or bronchiectasis. History of unstable angina, uncontrolled hypertension, or heart failure NYHA class 3-4. Overt clinical signs of right heart failure. Average resting systolic BP<110mmHg or average resting HR<60bpm. Any degree of heart block. Concomitant prescription of beta-blockers, rate-limiting calcium channel blockers, digoxin, or amiodarone. Patients must not have had an exacerbation receiving oral corticosteroids within the past 3 months. Short acting beta-2-agonist use (salbutamol) was allowed during the study, but patients withheld 6 hours prior to any visit.

Primary outcome: To establish effects between chronic dose exposure to cardio-selective and non-cardioselective beta blockers on airway resistance at 5Hz (R5) using IOS.

Secondary outcomes: IOS outcomes (R5, R20, R5-R20, X5, AX), spirometry (FEV_1, FVC, RVC), heart rate (HR), oxygen saturations (SpO2), blood pressure (BP), six minute walk test (6MWT), Brain Natriuretic Peptide (BNP), St George's respiratory questionnaire (SGRQ), he Baseline and Transition Dyspnoea Indexes (BDI-TDI), domiciliary spirometry, symptoms, HR and Sp02.

Data Analysis:

The study was powered on IOS at >80% to detect a 0·2 kPa/L.s difference in R5 with an SD of 0·23 kPa/L.s requiring a sample size of 18 completed patients per protocol using a cross-over design and alpha error of 0·05 (two-tailed). The data were checked for normality of distribution prior to analysis with both visual inspection of the histogram alongside utilisation of the Shapiro-Wilk Test of normality. Baseline values after run-in and washout were compared; having demonstrated no significant differences for treatment or sequence, the pooled baseline values were used for the purpose of subsequent comparisons with randomized treatments. Within and between treatment comparisons across visits were made by repeated measures analysis of variance (ANOVA) with time order effects assessed, and then, where a significant difference was observed, this was followed by post hoc pairwise testing with Bonferroni correction.

Effects of ultra-long acting bronchodilator therapy assessed by impulse oscillometry in smokers with asthma taking inhaled corticosteroids.

EudraCT No. 2014-005317-23

REC No. 15/ES/0032

Clinicaltrials.gov No. NCT02682862

Inclusion Criteria: Male or female volunteers aged 18-65 years with persistent asthma and on inhaled corticosteroids (at least 400 micrograms BDP or the equivalent daily); current smoker; FEV1 ≥ 60 % predicted.

Exclusion Criteria: Other respiratory diseases such as COPD, bronchiectasis or ABPA

Primary outcome: To assess the effects of once daily Striverdi Respimat® (olodaterol), versus Spiolto Respimat® (olodaterol-tiotropium), as add-on therapy to inhaled corticosteroids on airway resistance at 5Hz (R5), assessed by IOS.

Secondary outcomes: other IOS variables, spirometry, mannitol AHR, salbutamol recover post challenge, domiciliary PEF, ACQ.

Data analysis: The primary outcome of the study was change in airway resistance at 5Hz (R5). 16 completed subjects per protocol were required to detect, with 90%

power, a difference of 30% in R5 from baseline, comparing olodaterol alone with olodaterol-tiotropium, assuming a within subject SD of 24.5%, using a cross-over design, with alpha error of 0.05 [2 tailed].

Effects of add-on indacaterol and tiotropium to inhaled corticosteroid in non-smoking asthma

Primary Objective:

To compare single and chronic dosing protection against sensitivity (PD_{15}) and reactivity (RDR) to mannitol, for indacaterol alone versus indacaterol plus tiotropium given once daily as add-on therapy to pre-existing inhaled corticosteroids.

Secondary Objectives:

To compare single and chronic dosing of indacaterol versus indacaterol plus tiotropium on:

Bronchodilator response based on trough spirometry.

Airway resistance and reactance based on impulse oscillometry.

Asthma control questionnaire

Exhaled nitric oxide

Post challenge FEV_1 recovery

Abstract:

Background: Tiotropium is a long acting antimuscarinic (LAMA), licenced as triple therapy with inhaled corticosteroid and long acting beta-agonist (ICS/LABA). There may be a synergistic benefit between LAMA and LABA because of receptor crosstalk, which in turn could modify beta-2 receptor down-regulation and associated tolerance induced by LABA.

Objective: We hypothesise this mechanism may result in a reduction of airway hyperresponsiveness (AHR) when using triple therapy.

Methods: We evaluated 14 non-smokers with asthma using an open-label, randomized crossover design. ICS with Indacaterol and Tiotropium (IND/TIO) vs ICS with Indacaterol (IND) over 4 weeks with challenge performed after 1st and last doses at trough.

Results: We found no significant difference in mannitol sensitivity, expressed as the provocative dose of mannitol required to reach a 15% drop in FEV_1, or mannitol reactivity, expressed as the response dose ratio (RDR: max % fall in FEV1 / cumulative dose) , when comparing ICS/IND/TIO to ICS/IND. Geometric mean fold differences for RDR comparing single and chronic dosing were 3.26 fold (95%CI 1.46-7.29) and 2.51 fold (95%CI 1.32-4.79) for IND and IND/TIO respectively. Furthermore, salbutamol recovery post challenge was significantly blunted after chronic compared to single dosing with either ICS/IND ($P<0.005$) or ICS/IND/TIO ($P<0.05$).

Conclusion & Clinical Relevance: Our data suggests that concomitant tiotropium does not modify the bronchoprotective tolerance induced by Indacaterol, in turn suggesting that crosstalk may not be clinically relevant when using triple therapy. This study was registered on clinicaltrials.gov as NCT02039011.

Introduction:

Tiotropium (TIO) is a long acting muscarinic antagonist (LAMA), which is functionally selective for the post junctional M3 muscarinic receptor, found on airway smooth muscle (43). TIO reduces asthma exacerbations by 21% in patients when used as add-on therapy in patients receiving inhaled corticosteroids and long-acting beta-agonists (ICS/LABA)(59). Whilst blocking the M3 receptor inhibits acetylcholine induced bronchoconstriction, TIO exhibits only modest improvements in FEV_1, which amounts to approximately 100ml at trough (59, 60) ,which is less than the minimally important difference of 230ml (61). It is therefore hard to explain the protective effect on exacerbations on solely the basis of this small improvement in airway calibre alone (62).

One mechanism by which TIO may exhibit its protective effects is by attenuating airway hyperresponsiveness (AHR), via blockade of the post junctional M3 muscarinic receptor, resulting in reduced response to cholinergic transmission (152). M3, however, is not the only muscarinic receptor to contribute to increased airway tone and AHR; asthma is also associated with impaired pre-junctional M2 function (153) (41). The pre-junctional M2 is an inhibitory autoreceptor, as it is stimulated by acetylcholine to reduce further acetylcholine secretion. In asthma, the loss of this negative feedback mechanism results in increased AHR. Moreover, it has been

postulated that both pre-junctional beta-2 and M2 receptors are inhibitory to the release of acetylcholine and that there is crosstalk between these receptor types (41, 153). Hence it might be expected that chronic dosing with LABA might remove the brake to acetylcholine release as a consequence of down-regulation and subsensitivity of pre-junctional inhibitory beta-2 receptors, resulting in augmented cholinergic transmission and bronchoconstriction (153). In this regard, TIO rapidly dissociates from M2 receptors, unlike its affinity for post junctional M3 receptors, thereby facilitating additional inhibition by M2 receptors and reduced pre-junctional acetylcholine release. This functional M3 selectivity may be a possible mechanism by which it reduces exacerbations in asthma by attenuating AHR (43).

Another possible mechanism is that muscarinic M3 receptors promote beta-receptor desensitization through protein kinase C–mediated phosphorylation (115), hence inhibition of this effect by TIO may protect the beta-2 receptor from acetylcholine induced heterologous desensitization by acetylcholine(154). In this regard looking at the converse situation, tiotropium has been shown to protect against propranolol induced bronchoconstriction (155).

TIO may also reduce exacerbations via a putative anti-inflammatory action by inhibiting the paracrine effects of acetylcholine on inflammatory cells (156) . TIO has been shown to exhibit inhibitory effects on the development of airway remodelling in the animal model of antigen induced asthma(156, 157) . In vitro data have also suggested that there may be an anti-inflammatory synergy between LABA and LAMA, via the cAMP pathway (115).

Pointedly no studies have looked at effects of TIO on AHR assessed by bronchial challenge using non cholinergic agents. One study showed that, as expected, TIO produced prolonged functional antagonism of M3 mediated smooth muscle constriction induced by the cholinergic agonist methacholine (158). As TIO is only currently indicated as add-on therapy to ICS/LABA (45), the objective of this study was to evaluate the impact of adding TIO to ICS/LABA on AHR to mannitol, in patients with persistent asthma and whether TIO might also prevent against LABA induced subsensitivity (153).

Patients and Methods:

Non-smoking male or female patients aged at least 18 years, with persistent asthma already receiving ICS or ICS/LABA attended for a screening visit. Participants had to have a minimum FEV_1 of >50% predicted and be mannitol responsive i.e. provocative dose required to reduce FEV_1 by 15% (PD15) <635mg, to be enrolled. After initial screening, any LABA therapy was first withdrawn for 2 weeks followed by halving the ICS dose, to a minimum of 400µg/day (as beclometasone equivalent dose). If patients were on secondary controllers such as leukotriene receptor antagonists, these were also stopped. Participants then entered a 2-week run in on this dose of ICS, which was then continued throughout the study.

The trial was a single centre, randomised open label cross-over design. Patients received either 4 weeks of indacaterol (Onbrez Breezhaler, Novartis, Calberley, UK) alone at a dose of 150µg OD (IND), or combined with tiotropium (Spiriva

Handihaler, Boerhinger Ingelheim, Bracknell ,UK) 18µg OD (IND/TIO) as add-on to pre-existing ICS. There was a 2-week washout in between treatments while continuing to take the same dose of ICS. This washout was sufficient to minimise the possibility of carry-over effects of both IND and TIO (159).

Including screening, there were 7 visits in total (figure 2.).

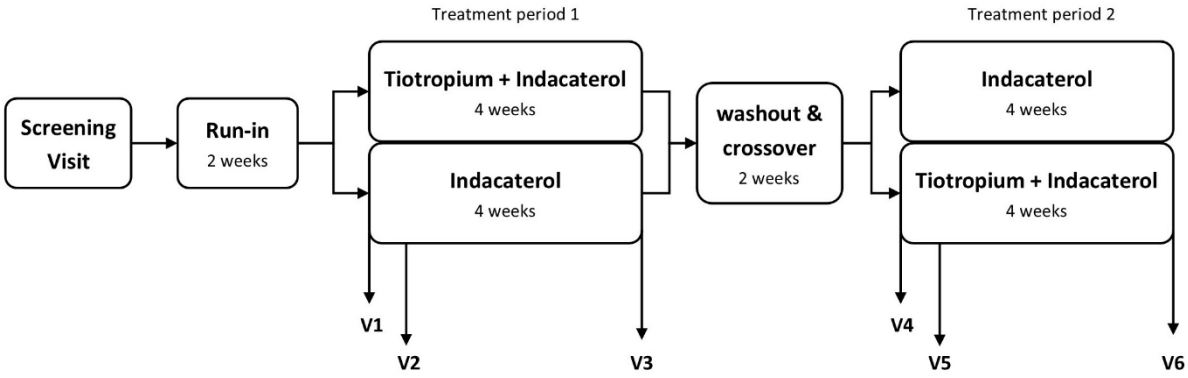


Figure 2. Flowchart

Visits were performed, in the mornings (8am to 10am), at baseline after run-in and washout, and at 24 hours (i.e. trough) after the first and last doses of each randomised treatment period. Patients were allowed short acting beta-2 agonists (SABAs) as a reliever during the study but were asked to abstain from SABA use at least 6 hours before each visit. . Participants were asked to record study medication use on a diary, and compliance was checked with returned empty capsule counts. This study was registered on clinicaltrials.gov as NCT02039011. The study was approved the Tayside committee for medical ethics (reference: 13/ES/0072) and full informed consent was obtained from all patients.

The primary outcome was mannitol challenge, specifically mannitol sensitivity as PD_{15}. Mannitol reactivity as the response dose ratio (RDR) was a secondary outcome. Mannitol challenge was performed as previously described(160). Impulse oscillometry, a secondary outcome, (Jaeger Masterscreen IOS) was performed as previously described (128) in accordance with manufacturer's guidelines. A SuperSpiro spirometer (Micro Medical Ltd) was used to perform spirometry in accordance with European Respiratory Society guidelines(161). After mannitol challenge, salbutamol (400µg) was administered and 30-minute recovery recorded. Exhaled nitric oxide (FeNO) was performed using an NIOX MINO analyser (Aerocrine AB), in accordance with the published guidelines (162). Asthma control questionnaire (ACQ-7) was measured using the standard 7 point paper questionnaire(146) (Qoltech, UK) .

Results:

The participant flow for the trial is shown in the consort diagram (Figure 3) below.

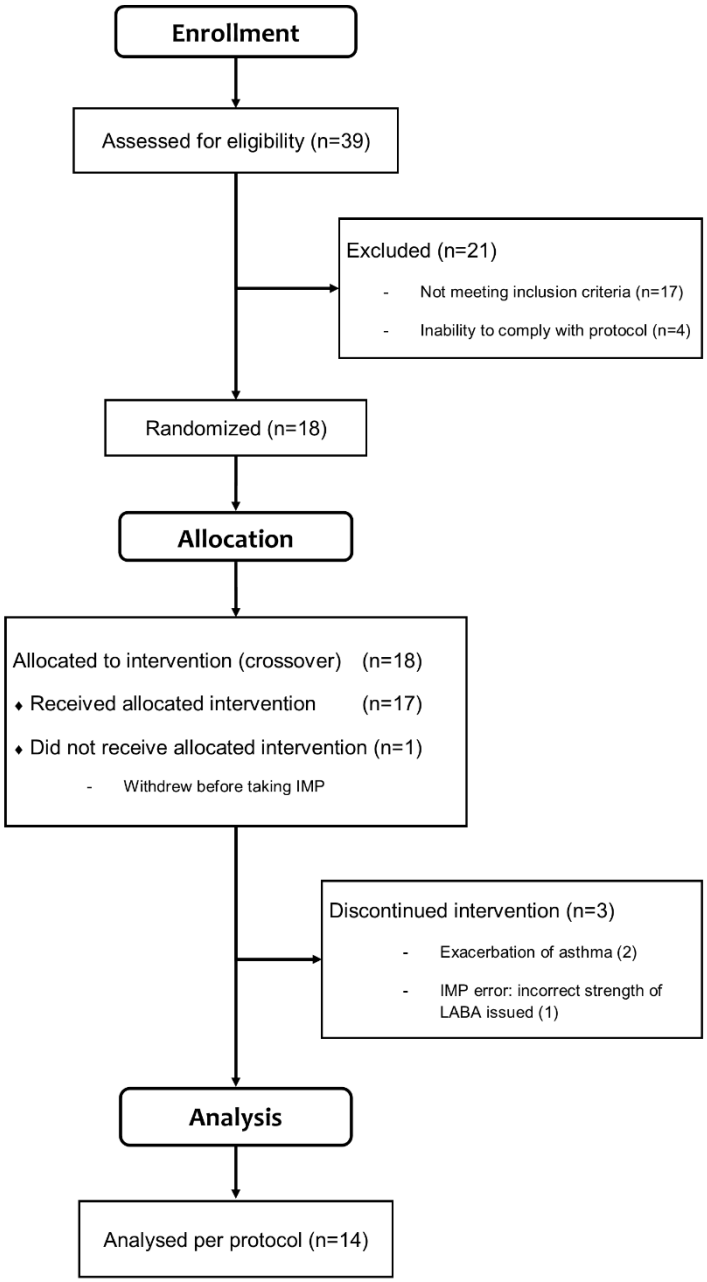


Figure 3. Consort

Of the 39 patients screened 18 were randomised and 14 completed per protocol. Of the 14 ICS treated asthma patients analysed, 12 had at least one positive skin prick test to common aeroallergens, mean age was 46 years , mean FEV_1 86% predicted, mean BMI 30kg/m^2, mean R5 160% predicted, and mean ICS dose 693µg/day (beclometasone equivalent dose). No patients were current smokers, two were ex-smokers with a mean pack year history of 2.6. Values comparing mean ICS dose pre and post step down were 693 vs 429 µg/day ($P< 0.05$).

Data for all outcomes according to study visits are summarised in Table 1. All outcome measures at first baseline and second baseline were assessed for carryover effect in order of sequence. There was no statistical difference between baseline data justifying the use of a pooled baseline value for comparison with randomised treatment arms. This confirmed an adequate washout period. In particular, there was no significant difference between mean baseline values for the primary outcome of mannitol PD15: 383mg vs 387 mg.

There were significant improvements ($P<0.05$) in mannitol PD15 and RDR with IND or IND/TIO vs baseline after single but not chronic dosing (Figure 4). There was a significant difference ($P<0.05$) in RDR between single and chronic dosing for both treatments: geometric mean fold differences were 3.26-fold (95%CI 1.46-7.29) and 2.51-fold (95%CI 1.32-4.79) for IND and IND/TIO respectively. Furthermore, salbutamol recovery post challenge was significantly blunted afterchronic compared to single dosing with either IND ($P<0.005$) or IND/TIO ($P<0.05$) (Figure 5 and table 1).

TABLE 1		**INDACATEROL**		**INDACATEROL + TIOTROPIUM**	
	Pooled baseline	**Single dosing**	**Chronic dosing**	**Single dosing**	**Chronic dosing**
FEV_1 (L)	2.56 (2.18-2.95)	2.69 (2.28-3.10)*	2.64 (2.26-3.02)	2.78 (2.38-3.19)*	2.71 (2.33-3.09)*
FEV1 Predicted (%)	87 (78-97)	91 (82-100)*	90 (81-100)	95 (85-105)*	93 (83-102)*
FEF_{25-75} (L)	1.79 (1.22-2.36)	2.02 (1.39-2.65)*	1.91 (1.25-2.57)	2.22 (1.49-2.95)*	1.94 (1.38-2.49)*
R5 (kPa/l.s)	0.54 (0.44-0.64)	0.45 (0.37-0.52)*	0.44 (0.37-0.50)*	0.39 (0.34-0.43)*	0.45(0.39-0.50)*
R5-R20 (kPa/l.s)	0.14 (0.07-0.22)	0.07 (0.03-0.11)*	0.07 (0.04-0.10)*	0.05 (0.03-0.07)*	0.08 (0.04-0.11)*
AX (kPa/l)	1.63 (0.58-2.68)	0.76 (0.34-1.19)*	0.68 (0.43-0.92)*	0.44 (0.25-0.63)*	0.78 (0.48-1.09)*
RDR (%/mg)	0.037 (0.025-0.055)	0.011 (0.005-0.026)*	0.037 (0.023-0.061)†	0.015 (0.008-0.029)*	0.035 (0.018-0.070)†
PD_{15} (mg)	390 (291-521)	537 (438-619)*	455 (342-606)	487 (329-624)*	388 (255-593)
FENO (ppb)	30 (20-45)	30 (20-44)	30 (20-45)	32 (23-45)	29 (19-44)
salbutamol Recovery (%.min)	47 (-79 - 172)	33 (-47 – 113)	259 (196 – 322)*	77 (19-136)	239 (177-300)*
ACQ7	0.72 (0.48-0.95)		0.44 (0.24-0.63)		0.50 (0.27-0.73)

Values are presented as mean (95% CI)
**Denotes significant (P<0.05) difference from pooled baseline.*
†Denotes significant difference (P<0.05) between single and chronic dosing within treatment groups.
No statistically significant differences observed between Indacaterol vs Indacaterol + Tiotropium when comparing single vs chronic dosing at trough. salbutamol recovery is expressed as the area under the curve (AUC) for 30 minutes.

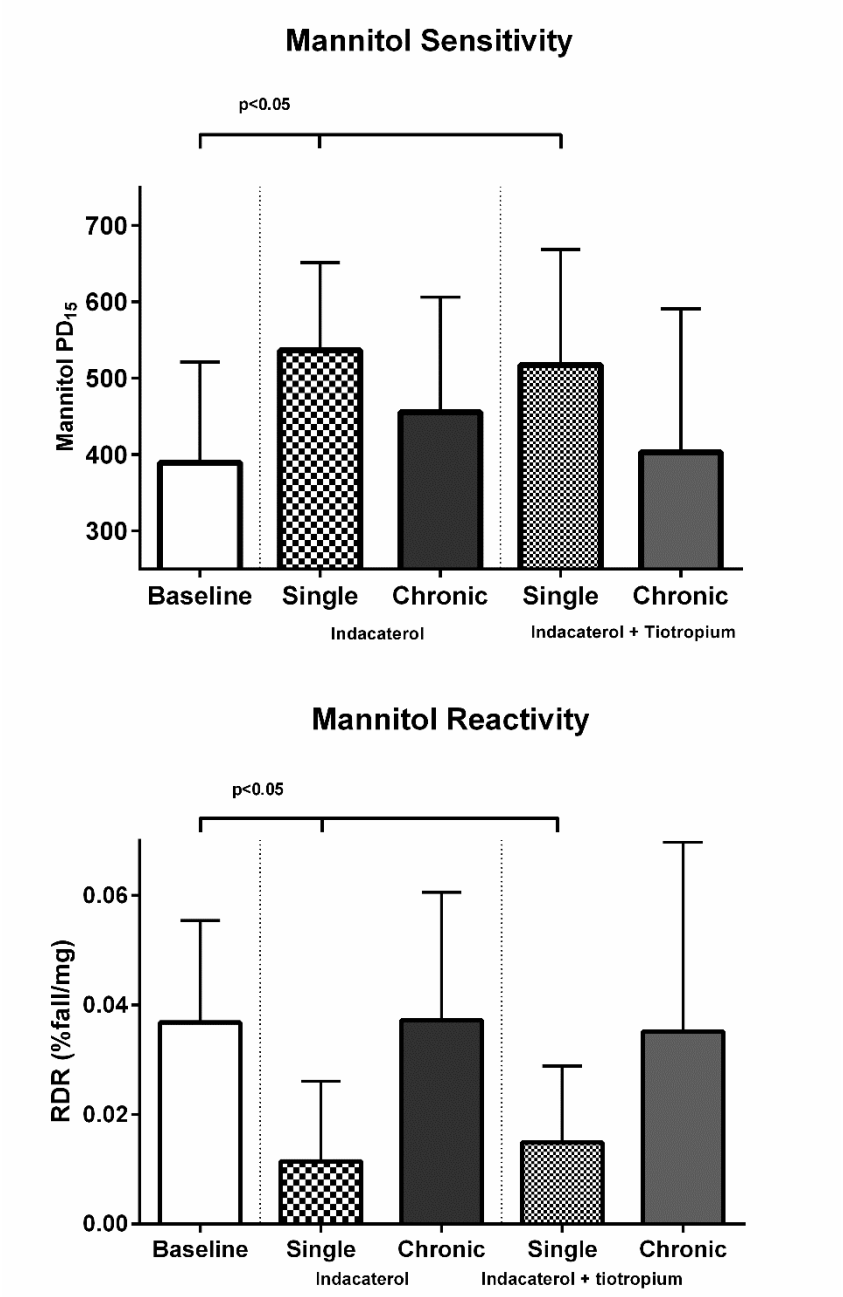


Figure 4.

Effects of randomized treatments (as add on to ICS) compared to baseline on (a) mannitol sensitivity (strength of stimulus) and (b) reactivity (responsiveness to stimulus). P value denotes significant difference for randomised treatments compared to baseline. There was also a significant difference between single and

chronic dosing for reactivity with both treatments. There were no differences between treatments. Values are geometric means and 95% CI.

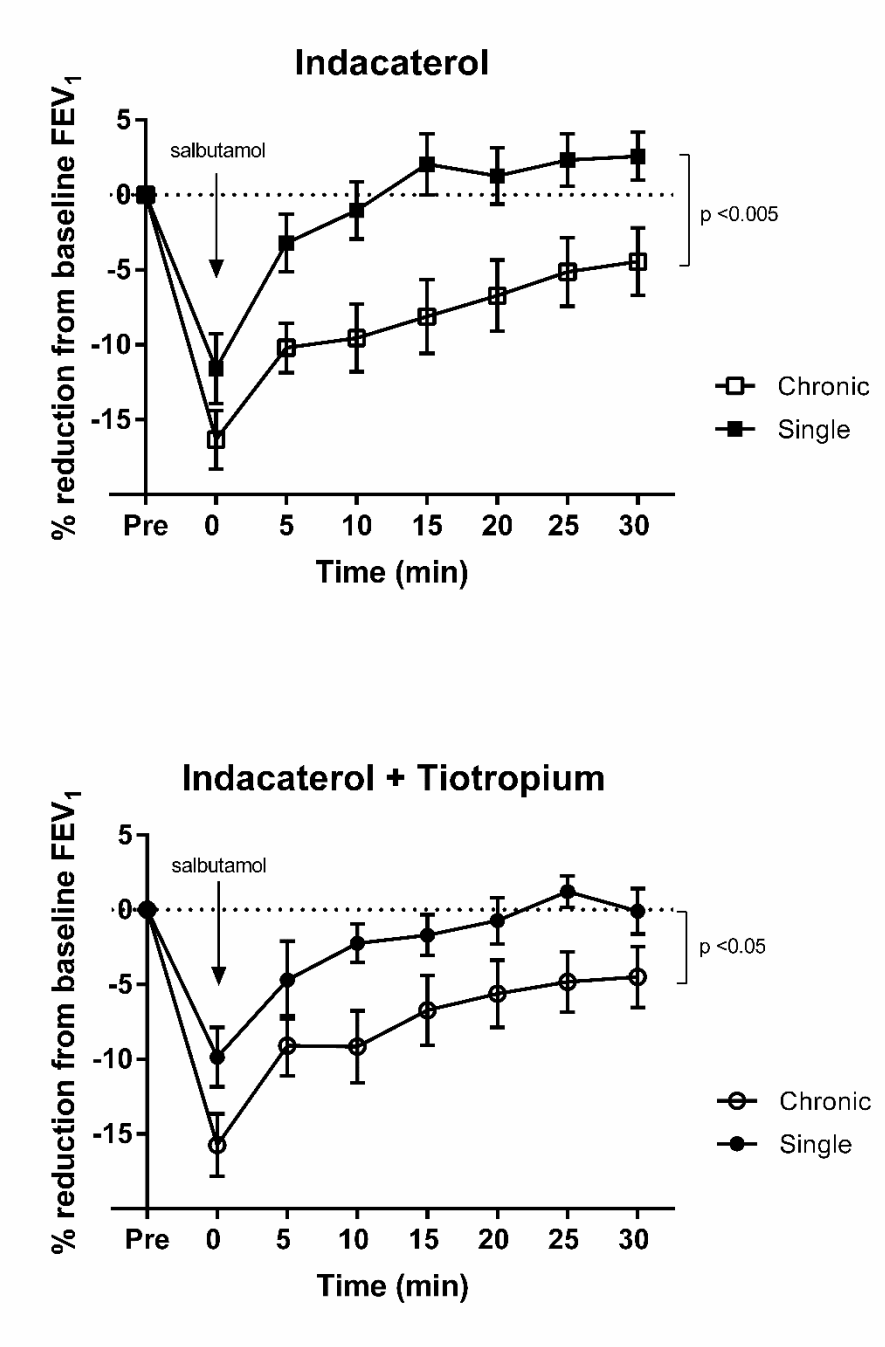


Figure 5.

Effects of single and chronic dosing with either (a) indacaterol alone or (b) indacaterol +tiotropium (as add on to ICS) on salbutamol (400ug) recovery post

challenge. P value denotes significant overall blunting of the salbutamol recovery comparing chronic vs single dosing. Values are means and SEM. Note not all patients achieved a 15% reduction in FEV_1 at the end of the mannitol challenge.

IOS measures including R5, R5-R20, and AX were all significantly improved ($P<0.05$) with both treatments compared to baseline after single and chronic dosing. FEV_1 and FEF_{25-75} were significantly better after single dosing with both treatments ($P<0.05$) but only after chronic dosing with IND/TIO ($P<0.05$). There were no significant differences between treatments after chronic dosing for either mannitol AHR, spirometry or IOS outcomes. FeNO was unchanged with either treatment compared to baseline. ACQ was also unchanged by either treatment.

Discussion:

Our results showed improvements in mannitol AHR with both treatments after single dosing which were not maintained after repeated exposure, in addition to blunting of salbutamol recovery. This is likely to be indicative of agonist induced down-regulation and uncoupling of beta-2 receptors and associated tolerance of response. The loss of bronchoprotection induced by indacaterol and associated cross tolerance seen as blunted salbutamol recovery has previously been well documented with other twice daily LABAs in patients taking concomitant ICS (163-165) (166) (49). Indacaterol has a high degree of intrinsic efficacy at the beta-2 receptor being 73% compared to the effect of isoprenaline in vitro.(167) In another study using isolated human bronchi the maximal relaxant response was 77% for indacaterol versus 94% for formoterol (168). In this regard prolonged stimulation with a high efficacy agonist like indacaterol would be expected to result in marked down

regulation and uncoupling of beta-2 receptors as has been previously shown with formoterol (165, 169-171). At 24 hours after the last dose of IMP (trough), the airway might be particularly vulnerable to exogenous constrictor stimuli immediately prior to the next dose. At this point we challenged the patients with mannitol to assess if the add on TIO resulted in less AHR. There was statistically no different with both treatments at day 1 in terms of degree of AHR, moreover this remained the case with chronic treatment. This also applied to recovery time post challenge.

Hence it can be concluded that we did not see any clinical evidence of crosstalk between muscarinic and beta-2 receptors, at least in terms of bronchoprotective subsensitivity using indirect challenge with mannitol (153). The absence of any bronchoprotection seen with TIO is consistent with similar findings with ipratropium using direct acting histamine challenge (172) . We chose to use mannitol challenge as it is a well validated (173) indirect challenge and hence better reflects other physiological stimuli than direct challenges such as methacholine or histamine. Furthermore, at the time of doing the study adenosine 5' monophosphate (AMP) for human use was not commercially available. Whilst it is noted that response to mannitol is influenced by ICS (92), our patients had to be mannitol responsive at the first visit whilst taking a stable ICS dose, which remained constant throughout the study. Therefore, we felt that any changes in mannitol AHR would only reflect the impact of bronchodilator treatments. Furthermore, the PD_{15} and RDR values were not statistically different between first and second baseline, suggesting no carryover effects between randomised treatment arms.

For IOS and spirometry, both treatments conferred improvements which were maintained after chronic dosing. As was the case with AHR, we found no significant differences in pulmonary function outcomes after chronic dosing comparing between IND/TIO and IND alone. This may reflect the relatively controlled asthma population we recruited that had only a mild degree of AHR at baseline (defined as a PD_{15} >155 mg) (174). Previous studies in more severe patients have shown that TIO in addition to ICS/LABA results in approximately 100ml improvement in FEV_1 (59), in turn suggesting that improved airway calibre per se is unlikely to be the explanation for reduced exacerbations(62) . We had originally considered that IOS might be more sensitive than spirometry at picking up subtle differences between double and triple therapy for bronchodilator effects measured at trough (175) (176).In the presence of a raised baseline R5 value of 160 % predicted, one might expect there to be plenty of room for further improvement comparing double and triple therapy, which was not the case. Further studies are indicated to look at whether IOS is more sensitive to effects of TIO in more severe patients.

There was no improvement in ACQ score which is unsurprising given the group was objectively well controlled; the mean baseline value of 0.72 being less than the 0.75 cut off value for optimal control(147). However, the failure of add-on therapy with LAMA to improve ACQ scores was also seen by Peters *et al* in a much larger and more severe cohort (109). FeNO was unchanged with either treatment, which could be explained by levels being already suppressed by concomitant ICS. Nonetheless, one would still expect the addition of TIO to have contributed to a modest further

reduction from a mean baseline value of 30ppb, as shown in another study in more severe patients looking at triple therapy (27).

The clinical relevance of our data is that when using triple therapy, at least in asthma, any effects of LAMA on exacerbations is unlikely to be due to bronchoprotective effects. Moreover, concomitant LAMA does not mitigate tolerance induced by LABA or cross tolerance to salbutamol. The caveat is that our patients only had mild to moderate asthma and hence we did not see any significant additive bronchodilator effects with LAMA. In other words, if LAMA had produced altered airway geometry then perhaps, we might have seen some additional bronchoprotection. Against this is the previous observation of Britton *et al* where ipratropium did produce a dose related bronchodilator response which was disconnected from any effects on AHR to histamine challenge (172).

We accept that our study has limitations in that our patients were initially well controlled Moreover; our sample size was not powered to detect additional bronchodilation with TIO. As airway geometry is an important determinant of bronchoprotection, our negative findings with TIO on mannitol challenge might simply reflect the lack of additional bronchodilator effect with TIO. Although we did not have a comparator limb with TIO alone, one would have expected to see additive effects on AHR after chronic dosing when the bronchoprotective effect of LABA had diminished, in terms of there being room for potential further improvement after the last dose. One could always argue that TIO is only indicated for use as add-on to ICS/LABA, as was the case in the present study, and hence performing a study

looking at TIO alone or in conjunction with ICS would have no clinical resonance. Finally, we acknowledge that we did not measure either sputum or blood eosinophils in the present study, although in that respect our patients were selected a priori based on AHR.

In conclusion, TIO did not modify the bronchoprotective tolerance induced by indacaterol or the cross tolerance seen on blunting of salbutamol recovery. Further studies perhaps involving bronchial biopsy might provide an insight into the putative anti-inflammatory action of TIO in asthma to help further elucidate the mechanism by which it reduces exacerbations in patients taking ICS/LABA.

Commentary:

In hindsight this study was perhaps too mild in terms of asthma entry criteria. The asthma population recruited were, on average, well controlled with mild AHR. Were it to be repeated an ACQ cut off of >1.0 (I.e. those without optimal control) should be selected. Another option would have been to recruit by severity of AHR, but this would perhaps be slightly artificial or contrived. More importantly, as recruitment for this and other studies proved a constant challenge, there was a realistic chance that being too picky would have resulted in an unfinished project. This project also took most of the three years to complete. This population is essentially in stark contrast to the tiotropium licensing studies- who were essentially poorly controlled asthmatics. There may an AHR signal present in the middle ground (moderate) asthma population, but it remains to be studied. Concepts from this study were taken forward (retaining AHR as a secondary outcome), the focus population became patients with asthma who smoke. The main primary outcome of the follow-on smoker

study became R5, mainly as it would align with the COPD study, but also because selecting patients on AHR would have been a challenging recruitment.

Effects of ultra-long acting bronchodilator therapy in smokers with asthma taking inhaled corticosteroids

Primary Objective

To assess the effects of olodaterol (LABA) alone versus olodaterol-tiotropium (LABA/LAMA) given once daily as add-on therapy to pre-existing inhaled corticosteroids using impulse oscillometry in smokers with asthma

Secondary Objectives

To assess the effects of olodaterol alone versus olodaterol-tiotropium given once daily as add-on therapy to pre-existing inhaled corticosteroids on:

- Spirometry
- Mannitol challenge: The provocative dose to cause this 30% increase in R5 (PD30)
- Asthma Control Questionnaire (7 point)

Abstract:

Background: Smoking worsens underlying asthma inflammation and induces resistance to inhaled corticosteroids (ICS). Small airways dysfunction measured by impulse oscillometry (IOS) is associated with worse control. Smoking asthmatics demonstrate enhanced response to a short acting muscarinic receptor antagonist, compared to their non-smoking counterparts and may benefit more from LAMA therapy than their non-smoking equivalents.

Objectives: We investigated the effects on small airways of adding long acting beta-agonist (LABA) alone or with long acting muscarinic antagonist (LAMA) to ICS in smokers with asthma.

Methods: 16 current smokers were enrolled: mean age 44 yr., FEV1 84%, FEF25-75 47%, R5 158%, ACQ 1.69, 20 pack yr. Patients were converted to a reference ICS as HFA-BDP during initial run-in at median dose of 800µg. Open label olodaterol 5µg od (OLO) or olodaterol 5µg /tiotropium 5µg od (OLO/TIO) was added to HFA-BDP for median duration of 3 week in a randomised cross over design, including run-in and washout periods on HFA-BDP. IOS and spirometry were measured after each treatment (BDP/OLO/TIO or BDP/OLO) and at baseline after run-in and washout (BDP).

Results: After chronic dosing IOS outcomes of resistance at 5Hz and reactance area under the curve (AX) and at trough were significantly improved with OLO/TIO compared to OLO. For the primary end point of total airway resistance (as R5) the mean difference (95%CI) at trough was: 0.06 (0.015-0.10) kPa/l/s, peripheral lung

reactance area (as AX) : 0.38 (0.08-0.68) kPa/l, whilst FEV1 was not different. These small airway improvements were reflected by significantly lower asthma control questionnaire score in the OLO/TIO group but not the OLO group.

Conclusions

ICS/LABA/LAMA was superior to ICS/LABA on trough small airway outcomes in asthma patients who smoke. The study was registered at clinicaltrials.gov as NCT02682862 and was approved by the East of Scotland Regional Ethics Committee (reference: 15/ES/0032).

Introduction:

Patients with asthma who smoke have significantly poorer asthma control compared to their non-smoking counterparts (101). Cigarette smoking in asthma is associated with a higher frequency of exacerbations and an increased number of life threatening asthma attacks, with asthma mortality greater among heavy smokers compared to those with asthma who never smoke (102, 103). This has a huge impact on health care resources due to unscheduled doctor visits and frequent hospital admissions. Despite this, smoking cessation rates are very low due to the highly addictive nature of tobacco smoking and the prevalence rate of smoking among the asthma population is similar to that of the general population (104).

People with asthma who smoke are particularly challenging to manage because they are resistant to the beneficial effects of inhaled corticosteroid (ICS), the mainstay of asthma treatment (105, 106). Smokers with asthma also have substantially greater

decline in FEV_1 over time versus non-smokers (107). The burden of morbidity extends beyond airflow limitation however, as cigarette smoking enhances airway hyperresponsiveness (AHR) independent of airflow obstruction (94-96). In terms of small airway dysfunction, it is known that smokers with early stage COPD are characterised by prominent small airway dysfunction (177), pointedly morphologic abnormalities are found in the small airways of asymptomatic cigarette smokers with normal conventional lung function (178, 179).

Unfortunately, there is no guideline consensus regarding how to best manage these patients. Therapeutic studies in asthma tend to exclude smokers because of concerns about recruiting patients with chronic obstructive pulmonary disease (COPD) (108); Several studies looking specifically at the efficacy of tiotropium in asthma excluded anyone who is a current smoker or those that have a pack year history of greater than 10 (59, 109, 110); and the most recent closed triple ICS/LABA/LAMA (beclometasone/ formoterol/ glycopyrronium) study in asthma also excluded current and former smokers along the same lines (180). Hence, there is an unmet need for therapeutic studies in those with asthma who continue to smoke.

Cigarette smoking may modify the determinants of airway inflammation in asthma, such as causing increased non-TH2 derived cascade. Sputum eosinophil counts are lower in asthmatic smokers compared to non-smoking asthma (105), smokers also have a greater degree of neutrophilia in their sputum, as well as increased levels of IL-8 both in sputum and epithelial cells (181). Smoking asthmatics demonstrate enhanced response to short acting muscarinic receptor antagonist, compared to

their non-smoking counterparts (112), with significantly greater synergistic bronchodilation with co-administration of short acting beta-2 receptor agonist. This in turn would suggest that asthmatics who smoke have enhanced cholinergic tone. In a small single dosing study of tiotropium added to ICS and long acting beta-2 agonist (LABA), in smoking vs non-smoking asthma, those with higher pack-years or lower baseline percentage FEV_1 showed greater increases in FEV1 in response to tiotropium (113).

In view of the non-TH2 derived airway inflammation and steroid resistance that smoking asthmatics exhibit, earlier use of synergistic long acting bronchodilator therapy may improve asthma control, as it is known that long acting muscarinic receptor antagonists (LAMA) confer specific anti-inflammatory properties. Costa et al have shown that combining the ultra-long acting $beta_2$ olodaterol with tiotropium gives synergistic benefit, in terms of anti-inflammatory response, with significant reductions in IL-6 and IL-8 levels (115) observed in vitro with human lung fibroblasts. Olodaterol and tiotropium also significantly restored cAMP levels in fibroblasts of asthma subjects, beyond the levels induced by the agents acting alone. This cAMP dependent signalling pathway provides negative feedback for inflammatory response.

Conventionally the long acting muscarinic receptor antagonist (LAMA) tiotropium is licensed as add on therapy to ICS/LABA in asthma. Kerstjens et al previously showed a 21% reduction in severe exacerbations with tiotropium on top of pre-

existing standard combination therapy in non-smoking asthma, along with a higher peak FEV_1 of 139mL (96-181 95%CI) (34, 182, 183).

In view of the above, we propose to assess the effects of olodaterol, a novel once daily ultra-long acting beta-agonist given alone versus olodaterol plus tiotropium in the form of Spiolto® Respimat®, a combination of once daily ultra-long acting beta agonist and muscarinic antagonist, as add-on therapy to pre-existing inhaled corticosteroids in smoking asthma using impulse oscillometry.

Although no previous studies have compared olodaterol-tiotropium to olodaterol via a single inhaler device such as we are proposing, a previous study has compared formoterol and tiotropium to formoterol alone (184), this demonstrated a statistically significant increase in 12 Hour FEV_1 in the formoterol-tiotropium group vs the single agent groups. We will measure airway resistance with impulse oscillometry (IOS). This is a non-effort-dependent pulmonary investigation that provides information about airway mechanics which is more sensitive than spirometry alone (185). Furthermore, IOS has previously been found to be a sensitive measure of small airways function in both asthma and COPD (175, 186). Smokers may have a greater degree of peripheral small airway dysfunction and the bronchodilator response may be detected using IOS.

Methods:

Currently smoking male or female participants, aged 18-65 years, were recruited from the NHS Scotland boards of Tayside and Fife, alongside our existing database

of asthma patients at the SCRR. Participants had to have persistent asthma and receiving inhaled corticosteroids (at least 400 micrograms BDP or the equivalent daily). Participants had to have an FEV1 ≥ 60% predicted, and were excluded if they had a diagnosis of COPD, or other clinically significant respiratory disease such as bronchiectasis, allergic bronchopulmonary aspergillosis etc. Participants should not have had an asthma exacerbation requiring steroids and/or antibiotics within one month of screening visit, or three months if they had been hospitalised due to their asthma. This study was registered at clinicaltrials.gov as NCT02682862, and was approved by the East of Scotland Regional Ethics Committee (reference: 15/ES/0032)

The trial was a single centre, randomised, open label, cross-over design. Participants received either 4 weeks of Olodaterol (Striverdi Respimat 5µg OD, Boehringer Ingelheim, Bracknell, Berkshire) or Olodaterol-Tiotropium (Spiolto Respimat 5µg OD, Boehringer Ingelheim, Bracknell, Berkshire), both in addition to Clenil Modulite. There was a 2-week washout between treatment periods. This washout was sufficient to minimise possibility of carry-over effects of both Olodaterol and Tiotropium.

After an initial screening visit with reversibility testing, eligible participants had LABA and LAMA (if applicable) withdrawn. Participants' ICS was rounded to equivalent Clenil BDP (a minimum of 400µg). Second line controller therapy such as cromones, or leukotriene receptor antagonists were permitted, with the proviso that participants withheld such therapies 72 hours prior to each visit. Participants were issued diary

cards to note symptoms, short acting beta agonist use, and peak expiratory flow (PEF). If PEF dropped below 70% of screening reference value, participants were advised to contact an emergency phone number carried by a study doctor. This would result in withdrawal from the study, and an immediate review. Patients were allowed SABA during the study but asked to withhold it at least 6 hours prior to any study visit.

Including screening there were five visits in total (figure 6). Visits were performed in the mornings (8am-10am). Participants attended at a therapeutic trough and received baseline impulse oscillometry (Jaeger Masterscreen IOS, Hoechberg, Germany), measuring parameters of R5, R20, X5, AX and Fres. Patients then completed standard spirometry (Super Spiro) after IOS was obtained. Participants had the first dose of IMP (either at treatment period one or two) in department.

The primary outcome of the study was change in airway resistance at 5Hz (R5).

Our previous study (183) had demonstrated no statistically significant difference in FEV_1 in terms of differences between ICS/LABA and ICS/LABA/LAMA. R5 was chosen as IOS is more sensitive than conventional spirometry (175). This primary outcome also aligned with that of the final COPD study within the thesis (187).

16 completed subjects per protocol were required to detect, with 90% power, a difference of 30% in R5 from baseline, comparing olodaterol alone with olodaterol-tiotropium, assuming a within subject SD of 24.5%, using a cross-over design, with alpha error of 0.05 [2 tailed].

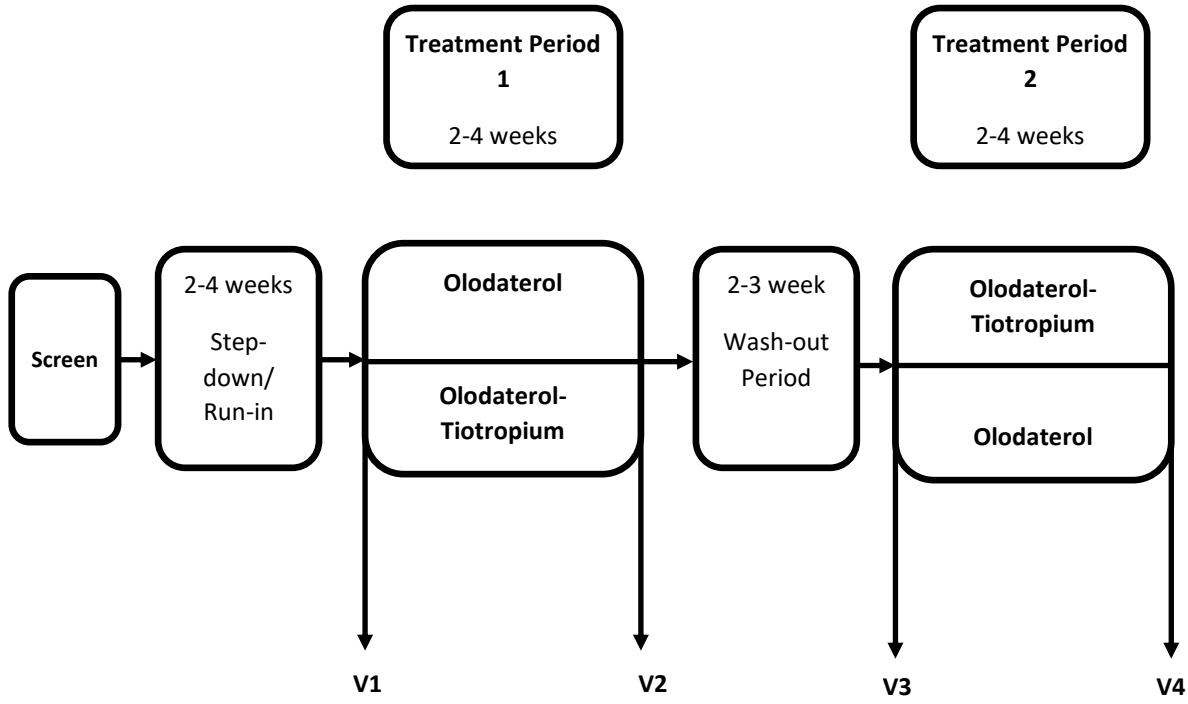


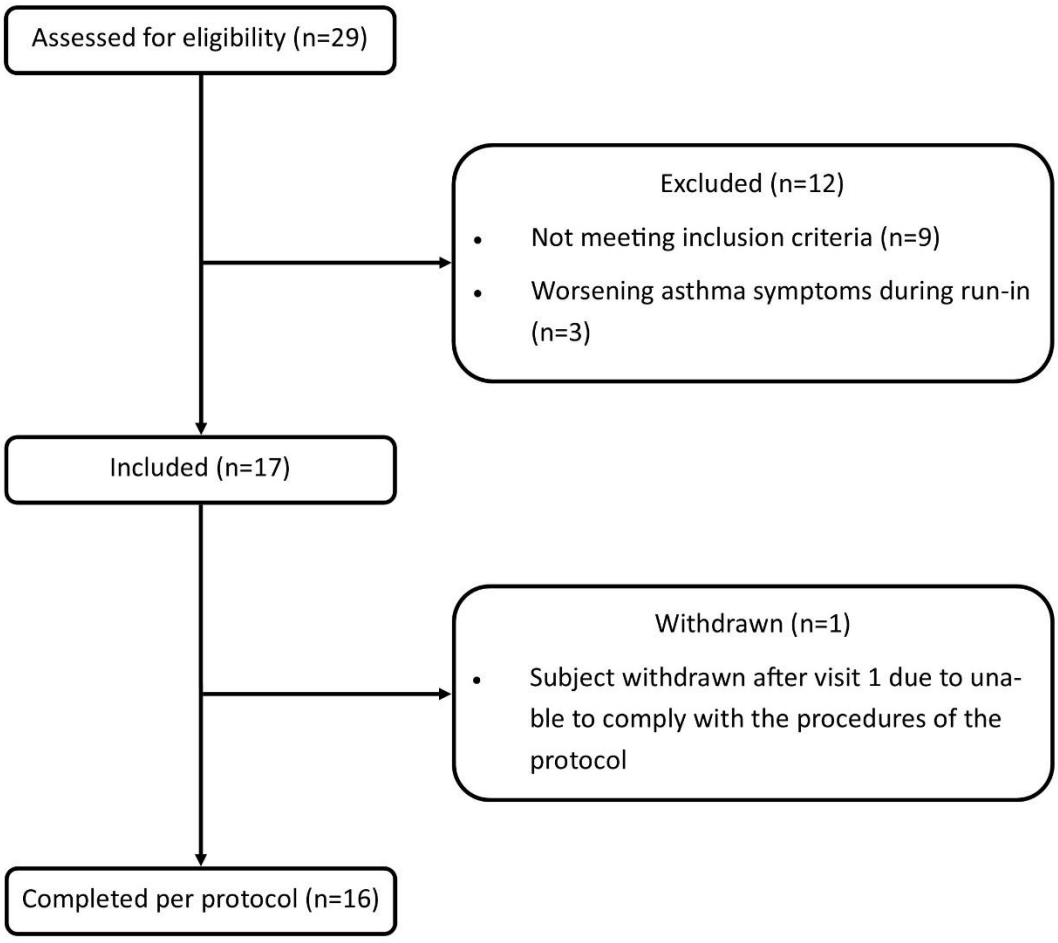
Figure 6. Flowchart

After one-hour post inhalation of IMP the IOS profile was recorded and the patient proceeded to a mannitol challenge (Osmohale, Pharmaxis Ltd, Sydney, Australia), per manufacturers guidelines. This was continued until an increase in R5 of 30% of baseline was achieved ($PD_{30}R5$). For patients whose R5 did not increase by 30% after 635mg, the maximum achieved increase in R5 was recorded. Once $PD_{30}R5$ or maximum increase in R5 was achieved, the participants were given 400 micrograms of salbutamol via an inhaler. To analyse recovery from the bronchial challenge, R5 was recorded every 5 minutes for 30 minutes, following the administration of salbutamol.

Results:

17 patients were randomised in order to complete 16 patients on a per protocol basis. One randomised patient was withdrawn due to a failure to comply with the protocol (figure 7).

Figure 7. Consort



Participants had a mean age of 44 years, FEV_1 of 84% predicted, FEF_{25-75} of 47% predicted, R5 of 158% predicted, ACQ of 1.69, a 20 pack years smoking history, and the median dose of HFA-BDP during run-in was 800 µg/day. 12/16 patients had at least one positive skin prick test with a median of 2 (IQR 2-4). The median duration for treatment, run-in and washout periods was 3 weeks. At respective baselines (per the crossover nature of the study) There were no differences in any outcomes comparing respective baseline values prior to treatment with OLO vs OLO/TIO (Table 2), hence baselines were pooled for analysis.

Table 2	**BDP/OLO**	**BDP/OLO/TIO**	**P value**
FEV_1 (L)	2.42 (0.18)	2.39 (0.74)	0.54
FEF_{25-75} (L/s)†	1.49 (0.17)	1.42 (0.18)	0.23
FVC (L)	3.49 (0.20)	3.49 (0.21)	0.98
R5 (kPa/l/s)	0.58 (0.03)	0.58 (0.04)	0.82
R20 (kPa/l/s)	0.42 (0.02)	0.41 (0.02)	0.55
R5-R20 (kPa/l/s)	0.16 (0.03)	0.17 (0.02)	0.32
AX (kPa/l)	1.64 (0.34)	1.98 (0.35)	0.19
fres (Hz)	18.70 (1.25)	20.79 (1.46)	0.13
X5 (kPa/l/s)	-0.23 (0.03)	-0.25 (0.03)	0.38

Baseline values for lung function prior to each randomised treatment. Values are presented as mean (SEM), † Geometric mean (SEM). BDP: Beclomethasone dipropionate, OLO: Olodaterol, TIO: Tiotropium. P value refers to comparison between baseline values

Primary outcome (R5):

Both olodaterol and olodaterol-tiotropium produced significant ($p<0.005$) reductions in R5 1-hour post dosing at the first visit and last visits:

At the first visit the changes in R5 with OLO and OLO/TIO were -0.14 (95% CI -0.20, -0.08), and -0.15 (95% CI -0.21, -0.09) respectively.

At the last visit the changes in R5 for OLO and OLO/TIO were -0.13 (95% CI -0.20, -0.07) and -0.15 (95% CI -0.21, -0.08).

There were no significant differences between OLO and OLO/TIO on R5 1-hour post dosing regardless of first visit or last visit.

The only difference found in primary outcome (R5) between OLO and OLO/TIO was at the last visit pre-dose. Here it was found that patients on OLO/TIO had lower airway resistance than OLO. At the last visit trough ΔR5 for OLO/TIO was -0.057 (95% CI -0.098, -0.015), vs OLO, with $P<0.005$ for difference.

Secondary outcomes:

Other impulse oscillometry measurements of R5-R20, X5, F_{res} and AX demonstrated similar statistically significant reductions at 1-hour dosing. There was no chance in R20 with either arm of the study (table 3). R5 (alongside above IOS parameters) were measured at chronic trough i.e. final visit, pre morning dose. In this regard there was a significant difference between olodaterol and tiotropium ($p=0.005$) ΔR5 olodaterol was -0.02 (-0.07, 0.39) ΔR5 olodaterol-tiotropium -0.07 (-0.13, -0.02). This was mirrored with AX, Fres and X5 (table 3).

Data for spirometry are presented in table 4. Both olodaterol and olodaterol/tiotropium significantly improved 1-hour post dose FEV_1 and FEV_1 % predicted at first dose and last dose compared to baseline, with no significant differences between therapies. This was the same for last visit pre-dose measurements. FEF_{25-75} demonstrated similar significant improvements at the 1-hour mark on first and last doses; however, did not improve compared to baseline at final visit pre-dose.

There was no significant difference between olodaterol or olodaterol/tiotropium in terms of mannitol challenge either expressed as (log_{10}) $PD_{30}R5$ or (log_{10}) RDR. The addition of either bronchodilator made no statistically significant difference in AHR compared to their respective ICS only baselines i.e. no statistically significant difference was noted between the ICS vs ICS/LABA and ICS vs ICS/LABA/LAMA.

Both olodaterol and olodaterol/tiotropium resulted in significant improvements in the seven domain ACQ score during the treatment period. Baseline ACQ score was established after an ICS only two-week run-in, a final visit ACQ was completed having received at least 2 weeks of the IMP. Olodaterol reduced ACQ from 1.72 (SD 0.85) to 1.29 (0.63) (p=0.046), olodaterol/tiotropium reduced ACQ from 1.53 to 1.03 (P=0.036). There were no significant differences between olodaterol and olodaterol/tiotropium in terms of ACQ, however only olodaterol/tiotropium improved ACQ to the MCID of 0.5 points.

TABLE 3

		OLODATEROL			OLODATEROL + TIOTROPIUM		
	Pooled baseline	**Peak (first dose)**	**Peak (last dose)**	**Trough**	**Peak (first dose)**	**Peak (last dose)**	**Trough**
FEV_1 (L)	2.40 (2.01 – 2.79)	2.64 (2.25 - 3.04)*	2.70 (2.30 – 3.10)*	2.52 (2.14 - 2.92)*	2.65 (2.22 - 3.08)*	2.74 (2.37 – 3.12)*	2.60 (2.21-2.99)*
FEV1 (%) Predicted	79 (73 - 85)	87 (81 – 93)*	89 (83 – 95)*	83 (78 - 89)*	87 (81 - 93)*	91 (84 - 98)*	86 (79-93)*
FVC (L)	3.49 (3.06– 3.92)	3.65 (3.20 – 4.10)*	3.71 (3.26 – 4.16)*	3.59 (3.15 – 4.03)*	3.63 (3.18 – 4.09)*	3.77 (3.34 – 4.20)*	3.69 (3.28 – 4.11)*
FEF_{25-75} (L/s)	1.62 (1.19 - 2.06)	1.97 (1.45 – 2.47)*	2.02 (1.48 – 2.56)*	1.62 (1.30 – 1.96)	2.03 (1.30 – 2.66)*	2.06 (1.49 – 2.64)*	1.72 (1.30 – 2.15)
R5 (kPa/l/s)	0.58 (0.51 – 0.65)	0.44* (0.37 – 0.51)	0.45* (0.37 – 0.52)	0.56 (0.47 – 0.66)	0.43* (0.37 – 0.49)	0.44* (0.36 – 0.51)	0.51*† (0.42 – 0.59)
R20 (kPa/l/s)	0.41 (0.38 - 0.45)	0.36* (0.32 – 0.40)	0.36* (0.32 – 0.41)	0.41 (0.38 – 0.45)	0.35* (0.31 – 0.39)	0.36* (0.31 – 0.40)	0.39 (0.32 – 0.44)
R5-R20 (kPa/l/s)	0.17 (0.11 – 0.22)	0.09* (0.05 – 0.13)	0.09* (0.04 – 0.12)	0.15 (0.08 – 0.22)	0.08* (0.04 – 0.12)	0.08* (0.04 – 0.12)	0.12* (0.06 – 0.17)
AX (kPa/l)	1.81 (1.13 – 2.49)	0.84* (0.35 – 1.34)	0.80* (0.31 – 1.28)	1.54 (0.69 – 2.39)	0.75* (0.30 – 1.21)	0.70* (0.23 – 1.16)	1.16*† (0.44 – 1.89)
Fres (Hz)	19.8 (17.2 – 22.3)	14.7* (12.2 – 17.2)	14.1* (11.4 – 16.8)	18.4 (15.1 – 21.7)	14.5* (12.0 – 16.9)	13.7* (11.2 – 16.2)	16.1*† (13.0 – 19.3)
X5 (kPa/l/s)	-0.24 (–0.31 – 0.17)	-0.17* (–0.22 – 0.11)	-0.16* (–0.21 – 0.11)	-0.21 (–0.28 – 0.15)	-0.15* (–0.20 – 0.10)	-0.15* (–0.19 – 0.10)	-0.19*† (–0.25 – 0.12)
ACQ7	1.63 (1.33 – 1.92)			1.29 (0.95 – 1.62)			1.03 (0.74 – 1.31)*

*Values are presented as mean (95% CI). *denotes significant ($p<0.05$) difference from pooled baseline. †denotes significant difference ($p<0.05$) between olodaterol vs olodaterol + tiotropium when comparing chronic vs chronic dosing at trough.*

Comparison between ex-smokers and smoker cohorts:

Our smoking cohort had similar ages and BMIs (p=ns) to our previously studied non-smoking cohort enrolled in our previous asthma trial, which also addressed the role of add on tiotropium to ICS/LABA treatment (183). The smoking asthma cohort also had similar baseline spirometry and impulse oscillometry values compared to the non-smoking asthma cohort. Specifically, no significant differences were found in the

TABLE 4

	Asthma group comparison	
	Non-smoking (n=14)	Smoking (n=16)
AGE (YEARS)	49 (15)	44 (11)
BMI (kg/m^2)	30 (7)	28 (5)
R5 (kPa/l/s)	0.54 (0.17)	0.58 (0.13)
R20 (kPa/l/s)	0.40 (0.09)	0.42 (0.07)
R5-R20 (kPa/l/s)	0.14 (0.13)	0.17 (0.10)
X5 (kPa/l/s)	-0.20 (0.10)	-0.24 (0.12)
FRES (HZ)	19.27 (6.86)	19.75 (4.77)
AX (kPa/l)	1.63 (1.82)	1.81 (1.28)
FEV1 (L)	2.56 (0.66)	2.40 (0.72)
FEV1 (% PRED)	87 (17)	79 (11)
FVC (L)	3.75 (0.85)	3.49 (0.81)

Data are presented as mean (SD). No significant difference observed (2-tailed, P<0.05) between smoking and non-smoking groups

IOS measures R5, R20, R5-R20, X5, AX, Fres, or in spirometry measurements of FEV_1, FEV_1 % predicted or FVC (table 4).

Discussion:

This study demonstrates that adding LAMA to ICS/LABA results in statistically significant improvements in the resistance measure R5, reactance measures X5, AX, and Fres, but not R20 and R5-R20, in the smoking asthma phenotype.

The study fulfilled its primary outcome in the sense that OLO/TIO patients had a lower R5 than OLO at the last visit trough. A therapeutic trough is a point where the airways are potentially most vulnerable to bronchoconstriction. However, the clinical significance of a 0.057 kPa/l/s reduction airway resistance (R5) noted with OLO/TIO is questionable, despite its significant P value. MCID for IOS parameters do not exist and its clinical use in day to day practice is still very much to be established.

These IOS measurements were more sensitive than conventional spirometry (FEV_1 and FEV_1 % predicted), which demonstrated similar statistically significant improvements in the ICS/LABA and ICS/LABA/LAMA groups, with no significant differences between the two arms of the study. Interestingly R5-R20 but not FEF_{25-75}, both thought to represent small airway function (128), failed to show any improvement with either ICS/LABA or ICS/LABA/LAMA at trough measurement. This may be because impulse oscillometry is an effort independent procedure and so less likely to be affected by poor patient technique.

At 1-hour post IMP dosing ICS/LABA/LAMA therapy was not significantly different to ICS/LABA in terms of any IOS measurement. The lack of any difference between randomised treatments for 1-hour post dose IOS response may be explained by the effect on airway calibre with olodaterol being maximal, with there being no further room for improvement with tiotropium. Alternatively it may relate to peak onset time of tiotropium, whilst tiotropium has an onset of action within 30 minutes, peak bronchodilation is reached within 3-4 hours (188). As visits were already 3-4 hours long, it was thought unreasonable to keep currently smoking patients in a controlled environment for an entire morning or more. Potentially the superiority at trough but not peak for IOS outcomes with add on tiotropium over ICS/LABA is due to a longer duration of action in small airways with tiotropium, which is evident at the end of the 24 hour dosing interval despite there being no difference in FEV1 (189).

The failure for R20 to improve with treatment may be in part due to its representation of large airway resistance. This may be indicative of the small airway disease asthma phenotype of the group who had a baseline R5-R20 of 0.17 kPa/l/s which is above the threshold for significant small airway resistance and correlates with poor asthma control (100).

This group had a mean ACQ of 1.63 (95% CI 1.33-1.92) suggestive of poorly controlled asthma (147). Both ICS/LABA and ICS/LABA/LAMA similarly improved ACQ above the MCID of 0.5 units. The lack of improvement in add on tiotropium reflects data already seen in the two largest replicate asthma trials which studied add on tiotropium to ICS/LABA in poorly controlled asthma (59). It is has been

suggested that improvement seen with add-on tiotropium to ICS/LABA in asthma on lung function and symptoms may be independent of T2 phenotype (26), however studied patients were non-smokers. Our hypothesis that add on LAMA may confer symptomatic benefit to smokers with asthma, who may have additional non-type 2 airway inflammation was not proven clinically. Had a difference been observed between ICS/LABA vs ICS/LABA/LAMA in ACQ, the open label nature of the study would no doubt have been a confounding factor.

Previously we have demonstrated that the addition of tiotropium to ICS/LABA confers no benefit to AHR in non-smoking asthma patients (183). We hypothesised that there may be a lack of sensitivity to AHR when measured against the conventional spirometry index of FEV_1. An exploratory outcome of AHR measured against R5 demonstrated no difference between ICS/LABA and ICS/LABA/LAMA, this was when expressed as the PC_{30} of R5 (sensitivity of the airway) and the RDR (reactivity of the airway) to mannitol. No signal could be discerned using R5 against mannitol, and the study was, in retrospect, too small to attempt to explore this outcome.

The study is limited by a number of issues. The study was carried out 'open label' , but the IMPs were delivered via the same Respimat device to eliminate the confounding factor of inhaler technique. Olodaterol-tiotropium Respimat is not licensed for asthma, whilst tiotropium Respimat is the only licensed LAMA for asthma (at the time of writing). We accept the treatment period of 2-4 weeks may be considered short; however, this is justified to some extent by a previous 13-week

tiotropium treatment study. This demonstrated that improvements in pulmonary function reached steady state at week one of a 13 week treatment period (190). The study did not assess any other small airways measurement such as multiple breath nitrogen washout, whole body plethysmography, nor was any lung imaging performed. Finally, the trial duration was not sufficient to assess whether exacerbation risk was reduced in those receiving add on LAMA. Whilst it is known that tiotropium reduces exacerbations in poorly controlled non-smoking asthma(182), no such data exists for smokers.

We previously performed a study in non-smoking asthma patients comparing ICS/LABA or ICS/LABA/LAMA (183) and observed significant improvements compared to a baseline of ICS alone for R5, R5-20 and AX, but no differences between randomized treatments. Whilst the patients had similar baseline demographics, spirometry and impulse oscillometry, the smoking patients in the present study had worse asthma control in terms of ACQ (0.72 vs 1.69). These patients may mimic the asthma-COPD overlap phenotype, in particular with regards to greater LAMA responsiveness (191). A small study assessing the bronchodilator effect of tiotropium in smoking and non-smoking asthma has previously demonstrated greater improvements in FEV_1 post dose in the smoking cohort versus the non-smoking cohort, all of which were receiving baseline ICS. This study only assessed a single dose of tiotropium, and patients were not receiving LABA (113), moreover no small airway indices were measured.

It is well known that smokers with asthma are resistant to the beneficial effects of inhaled corticosteroids (106). Our patients were on a median ICS dose of 800µg BPD, which would be considered at least a moderate dose (6). Future studies could assess whether ICS dose reduction to low dose, whilst patients receive LABA/LAMA could provide acceptable asthma control in smoking asthma, whilst reducing corticosteroid exposure. This could be evidenced both by stable spirometry, impulse oscillometry and ACQ score. It perhaps is worth emphasising still that smoking cessation is the best treatment any current smoker with asthma could undertake.

In conclusion this is the first study to demonstrate small airway improvements at trough in smokers with asthma receiving ICS/LABA/LAMA vs ICS/LABA. Assessment of small airways using impulse oscillometry delineated an improvement not detected via conventional spirometry. These improvements corresponded to a reduction in ACQ greater than the MCID of 0.5. Further studies are warranted to assess whether the addition of LAMA to ICS/LABA can result in a reduction of ICS doses in smoking asthma, or whether such small airway improvements are the reason for reduction in exacerbation risk.

Commentary:

These patients were more severe asthmatics than the previous study, clearly evidenced by their ACQ. The clear improvement seen in ACQ was perhaps only modestly represented by the positive primary outcome. The change, whilst statistically significant, was numerically minute. Compound this with the fact that no MCID exists for IOS, it is hard to ascertain how to take this finding forward. In truth a greater magnitude of change in IOS may have been seen if we had asked

patients to wait for a 4-hour post dose (the true peak of TIO) IOS measurement. This would have made an existing 4 hour visit even longer or added another visit onto the study. The IOS challenge proved to be both technically challenging and revealed no interpretable results. The patients were not selected to have AHR as it was not the primary outcome, therefore we enrolled a proportion (approximately 20%) of patients who were unresponsive to mannitol at the outset.

Beta-Blocker Therapy in Moderate to Severe COPD.

Abstract

Background: Beta-blockers remain underused in patients with COPD and cardiovascular disease for fear of bronchoconstriction.

Aim: We compared how different bronchodilator therapies affect tolerability of bisoprolol and carvedilol in moderate to severe COPD.

Design: A randomized, open label, cross-over study.

Methods: We compared the cardiopulmonary interactions of bisoprolol 5mg qd or carvedilol 12·5 mg bid for 6 weeks in conjunction with: (a) triple: inhaled corticosteroid /long acting beta-agonist/long acting muscarinic antagonist (ICS+LABA+LAMA), (b) dual: ICS+LABA, (c) ICS alone.

Results:18 patients completed, all ex-smokers, mean age 65 years, forced expiratory volume in 1 second (FEV1) 52% predicted. Bisoprolol and carvedilol produced comparable significant reduction in resting and exercise heart rate. FEV1, forced vital capacity (FVC) and lung compliance (AX) were significantly lower with carvedilol vs bisoprolol while taking concomitant ICS/LABA ($P<0·05$) but not ICS/LABA/LAMA.

Conclusions: In summary, bisoprolol was better tolerated than carvedilol on pulmonary function at doses which produced equivalent cardiac beta-1 blockade. Worsening of pulmonary function with carvedilol was mitigated by concomitant

inhaled LAMA (tiotropium) with LABA (formoterol), but not LABA alone. Registered at clinicaltrials.gov: NCT01656005.

Introduction

Beta-adrenoceptor (β-ADR) antagonists are indicated in guidelines for the treatment of heart failure and post myocardial infarction (192).There is a reticence to use beta-adrenoceptor antagonists in patients with COPD, as they are perceived as contraindicated (137). So called cardioselective antagonists such as bisoprolol have a 14 fold higher affinity for β1 than β2-ADR, while non selective antagonists such as carvedilol have a much higher relative affinity for β2-ADR(193). In heart failure, 40% of cardiac β-ADR are of the β2 subtype due to a relative downregulation of cardiac β1ADR from increased sympathetic drive (194). Hence one might postulate that preferentially blocking only β1-ADR might not be as effective as blocking both subtypes. In one study comparing carvedilol and metoprolol in heart failure with reduced ejection fraction, the former was associated with 17% lower mortality, with similar reductions in heart rate(195). Metoprolol is less selective than bisoprolol with a 2 fold higher affinity for β1/ β2-ADR(193).

The presence of β2ADR antagonism may result in bronchoconstriction. Management guidelines clearly reinforce the use of cardioselective beta-blockers in patients with heart failure and chronic obstructive pulmonary disease (COPD) (196, 197). Despite this, lower prescribing rates for beta-blockers have been described in

COPD patients with heart failure or post myocardial infarction (119, 136-138). In a meta-analysis of 15 retrospective studies in 21,596 patients with COPD the pooled estimate for mortality reduction with beta-blockers was 28% and for exacerbations was 38% (198). Among patients with known coronary arterial disease the mortality reduction was 39% and in heart failure was 26%. However, it remains unclear if beta-blockers may reduce exacerbations or mortality in individuals with COPD who have *covert* (untreated or unrecognized) cardiovascular disease (199). Prior to assessing this, which would require a longer study, a prospective trial is required to address concerns over potential beta blocker induced bronchoconstriction and whether conventional inhaler therapy confers bronchoprotection.

We performed a study comparing two commonly used selective and non-selective beta blockers, evaluating their impact on spirometry, impulse oscillometry, vital signs, exercise, and symptoms. This was in patients with moderate to severe COPD using realistic doses of bisoprolol 5mg od and carvedilol 12·5mg bd, the most commonly tolerated doses in real life older populations (200).

In particular we assessed how their pulmonary tolerability was impacted by inhaled bronchodilator therapy, namely when patients were on ICS/LABA/LAMA, ICS/LABA and ICS alone. Namely LABA: formoterol, 24 micrograms; LAMA: tiotropium 18 micrograms; and ICS: beclometasone dipropionate, 400 micrograms.

Once patients achieved a steady state on their respective beta blockers they underwent a sequential step down inhaled therapy: starting with ICS/LABA/LAMA, ICS/LABA and finally ICS alone – this sequence allowed us to dissect out the

respective interactions between beta-blockers with LAMA (i.e. ICS/LABA/LAMA vs ICS/LABA) and LABA (i.e. ICS/LABA vs ICS). It should be noted that whilst ICS only therapy is not licensed in COPD, in real life 8% of patients are taking such treatment (119). Our hypothesis was that cardioseletive beta blockers would be well tolerated and have little impact on pulmonary function values and symptoms. Non-selective beta blockers were expected to have a degree of negative impact on pulmonary function and symptoms. It was uncertain what the impact of bronchodilators would be. The reason to step up rather than step down was based on the hypothesis that the bronchodilators are conferring bronchoprotection against a “challenge” such as a non-selective beta blocker. This may aid clinicians in deciding which types of beta blocker to prescribe in the real world i.e. a cardiologist or general physician may not go about adding inhaled therapy to a patient with moderate to severe COPD, but may be more comfortable knowing patients on ICS/LAMA/LAMA can tolerate any beta blocker at a therapeutic dose.

A unique feature of our study was to use IOS to measure frequency dependent lung resistance (R) and compliance (as its reciprocal reactance: X). IOS is thought to be more sensitive than measuring forced expiratory lung volumes and flow rates using spirometry in order to detect subtle differences in airway constriction or dilatation (201). For this reason, total airway resistance (R5) was chosen as the primary outcome.

Methods

Patients aged 40-80 with moderate to severe stable COPD, GOLD stages 2 and 3 (197), were initially screened, with FEV1 30-80% predicted, FEV1/FVC ratio <0·70 smoking history of >10 pack years, and oxygen saturation ≥92% on room air, in sinus rhythm.

The primary outcome was total airway resistance (R5). The study was powered on IOS at >80% to detect a 0.2 kPa/L.s difference in R5 with an SD of 0.23 kPa/L.s requiring a sample size of 18 completed patients per protocol using a cross-over design and alpha error of 0.05 (two-tailed)

Visits were carried out between 8am-10am. Measures at each visit comprised impulse oscillometry (Jaeger Masterscreen IOS, Hochberg, Germany); Spirometry performed to British Thoracic Society standards (SuperSpiro, Micro Medical Ltd, Chatham, Kent, United Kingdom); St George's respiratory questionnaire (SGRQ) for health status and transition dyspnoea index (TDI) were also recorded. A 6-min walk test (6MWT) was performed (202) with heart rate, blood pressure, oxygen saturation (SpO2) and modified Borg scale for dyspnoea and fatigue, all recorded pre and post exercise. Blood was taken for serum potassium, blood eosinophils, and serum N-terminal pro-BNP.

A randomized cross-over open label design was employed (Figure 8) comprising eight visits in which patients received 6 weeks of either carvedilol or bisoprolol. There was a 2-week washout between treatment period one and two, to ensure at least a 5 half-life washout between beta-blocker treatment arms.

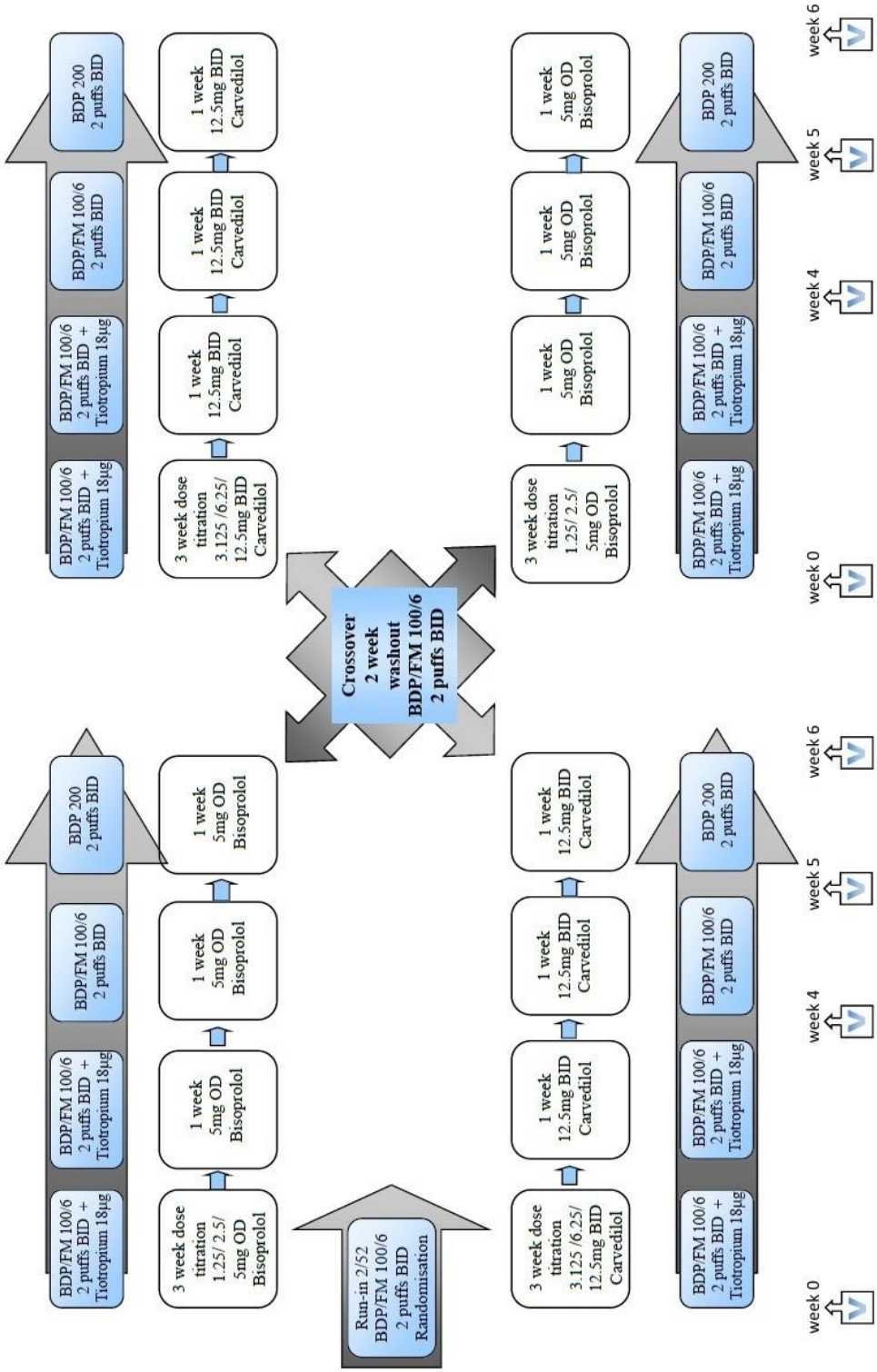


Figure 8. Study design.

Results:

The participant flow for the trial is shown in the below consort diagram.

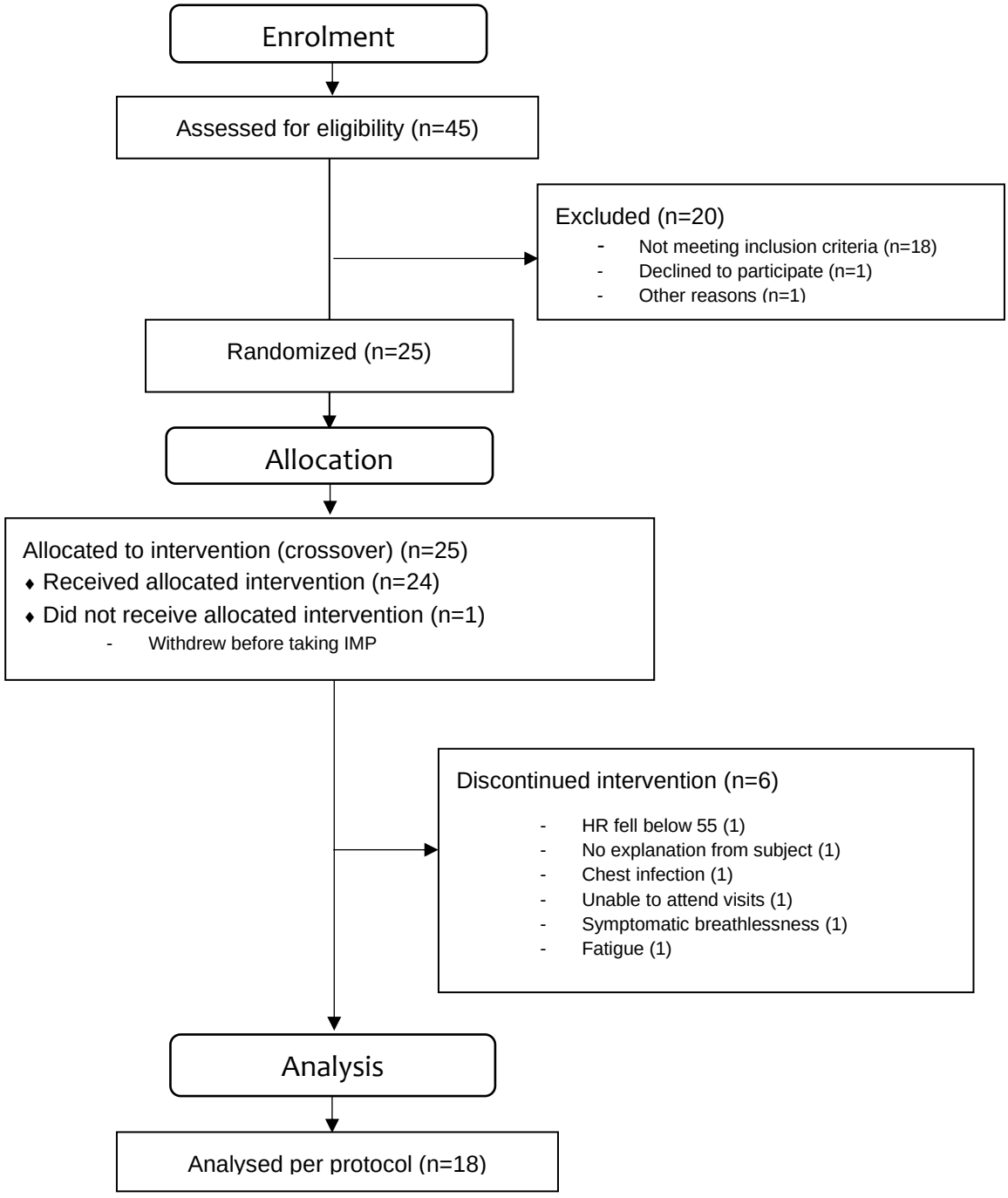


Figure 9. Consort

The trial ended when 18 patients completed per protocol. 83% of patients were male, with a mean age of 65 years, all were ex-smokers, Caucasian, with a mean pack year history of 47; FEV_1 52% predicted, FVC 93% predicted, FEV/FVC ratio 0·45. All patients were on at least one long acting bronchodilator (either LABA or LAMA). 72% of patients were receiving concomitant ICS therapy, with a median BDP-equivalent daily dose of 400µg.

The mean sequential reversibility in FEV1 was 7% (100ml) after 15 min following 400ug salbutamol and 12% (180ml) after 45 min following 80ug of ipratropium. Mean NT pro-BNP levels were modestly elevated at 308pg/ml (95%CI 194-445) with an age independent value of <300 pg/ml generally accepted as normal (203). Mean blood eosinophil count was 210 cells/µL.

Pulmonary function:

Respective baseline pulmonary function values after run-in and washout (i.e. on ICS/LABA but no beta-blocker) were not significantly different (Table 5), justifying the use of a pooled baseline for comparisons with randomized treatments.

IOS: Higher AX values indicate reduced lung compliance. A higher AX value was associated with carvedilol use compared with bisoprolol in all arms of the study, regardless of inhaled therapy. The increase in AX observed with bisoprolol treatment and ICS alone, was blunted by the addition of LABA or LABA/LAMA.

Table 5

	Bisoprolol	Carvedilol
FEV_1 (L)	1.52 (1.27-1.85)	1.47 (1.25-1.76)
FEV1 Predicted (%)	52 (46-60)	51 (44-58)
FVC(L)	3.41 (2.93-3.95)	3.39 (2.90-3.91)
RVC (L)	3.56 (3.04- 4.12)	3.56 (3.06-4.09)
R5 (kPa/l.s)	0.73 (0.56-0.93)	0.61 (0.52-0.70)
R20 (kPa/l.s)	0.40 (0.34-0.48)	0.38 (0.33-0.43)
X5 (kPa/l.s)	-0.30 (-0.38- 0.23)	-0.29 (-0.37-0.21)
AX (kPa/l)	3.09 (2.22-4.04)	2.92 (1.48-2.57)
HR (bpm)	76 (70-83)	74 (70-80)
Systolic BP (mmHg)	133 (126- 140)	136 (130- 143)
Diastolic BP (mmHg)	79 (75- 84)	82 (77-87)

Respective baseline values prior to randomized treatment with either bisoprolol or carvedilol. There were no significant differences between pre beta-blocker baselines (p=>0.05 all comparisons).

When receiving ICS/LABA/LAMA no statistically significant difference was noted in AX upon exposure to carvedilol (2.93 kPa/l) or bisoprolol (2.6 kPa/l), p=ns. When LAMA was withdrawn a statistically significant difference was noted between carvedilol (3.86 kPa/l) vs bisoprolol (2.86 kPa/l), p=<0.05.

Similarly, when receiving ICS/LABA/LAMA no statistically significant difference was noted in X5 upon exposure to carvedilol (-0.28 kPa/l.s) or bisoprolol (-0.25 kPa/l.s), p=ns. With withdrawal of LAMA there was a significant difference in X5 comparing bisoprolol (-0.28 kPa/l.s) vs carvedilol (-0.36 kPa/l.s), p=<0.05 (Figure 9 and Table

6). No differences were found in either R5 or R20 comparing carvedilol to bisoprolol at any level of inhaled therapy (ICS/LABA/LAMA vs ICS/LABA vs ICS).

Spirometry: There was no difference between FEV_1 between carvedilol or bisoprolol when patients were on ICS/LABA/LAMA. This was also the case for FVC (Figure 10 and Table 6). When LABA was withdrawn FEV_1 was significantly reduced in patients receiving carvedilol (1.37L) compared to bisoprolol (1.49L) $p=<0.05$. The differences in FEV_1 were above the minimal clinically important difference (MCID) of 100mls at trough (34). This was similarly noted in FVC with significantly lower FVC in carvedilol (3.32L) vs bisoprolol (3.57L) with withdrawal of LAMA, $p=<0.05$.

With withdrawal of LABA (ICS monotherapy) FVC was significantly lower in carvedilol (2.97L) vs bisoprolol (3.17L), $p<0.05$.

Table 6		ICS/ LABA/LAMA	ICS/ LABA/LAMA	ICS/ LABA	ICS/ LABA	ICS	ICS
	Pre beta-blocker	**Bisoprolol**	**Carvedilol**	**Bisoprolol**	**Carvedilol**	**Bisoprolol**	**Carvedilol**
FEV_1 (L)	1.50 (1.20-1.79)	1.53 (1.21-1.84)	1.47 (1.16-1.78)#	1.49 (1.21-1.84)‡	1.37 (1.06-1.68)*†‡	1.34 (1.03-1.65)*	1.26 (0.94-1.59)*
FVC(L)	3.40 (2.83-3.97)	3.58 (2.98-4.19)	3.48 (2.90-4.07)	3.57 (2.94-4.21)‡	3.32 (2.74-3.90)†‡	3.17 (2.56-3.77)*	2.97 (2.38-3.57)*†
RVC (L)	3.56 (2.98-4.14)	3.73 (3.14-4.33)	3.67 (3.06-4.28)	3.75 (3.19-4.31)	3.54 (2.95-4.12)‡	3.37 (2.81-3.93)	3.16 (2.57-3.75)*
R5 (kPa/l.s)	0.67 (0.52-0.82)	0.58 (0.47-0.69)	0.61 (0.51-0.71)	0.62 (0.51-0.73)	0.69 (0.58-0.80)	0.68 (0.59-0.77)	0.71 (0.60-0.81)
R20 (kPa/l.s)	0.39 (0.34-0.45)	0.38 (0.32-0.44)	0.37 (0.32-0.41)	0.39 (0.34-0.44)	0.38 (0.33-0.44)	0.37 (0.34-0.41)	0.39 (0.33-0.44)
R5-R20 (kPa/l.s)	0.28 (0.17-0.38)	0.21 (0.13-0.28)	0.24 (0.17-0.32)	0.23 (0.16-0.31)	0.31 (0.22-0.39)	0.31 (0.23-0.38)	0.32 0.245-0.40)
X5 (kPa/l.s)	-0.29 (-0.37-0.21)	-0.25 (-0.34-0.17)	-0.28 (-0.37-0.20)	-0.28 (-0.35-0.21)	-0.36 (-0.44-0.27)*†	-0.35 (-0.47-0.23)	-0.38 (0.46-0.30)*
AX (kPa/l)	3.01 (2.05-3.99)	2.60 (1.14-3.79)	2.93 (1.92-3.94)#	2.86 (1.80-3.91)‡	3.86 (2.75-4.97)*†	3.89 (2.80-4.98)*	4.30 (3.34-5.25)*

Pooled baseline values (i.e. pre beta-blocker) are shown while taking ICS/LABA after run-in. *P<0.05 carvedilol or bisoprolol vs baseline; †P<0.05 carvedilol vs bisoprolol. ‡P<0.05 ICS/LABA vs ICS for either beta-blocker, #P<0.05 ICS/LABA/LAMA vs ICS/LABA

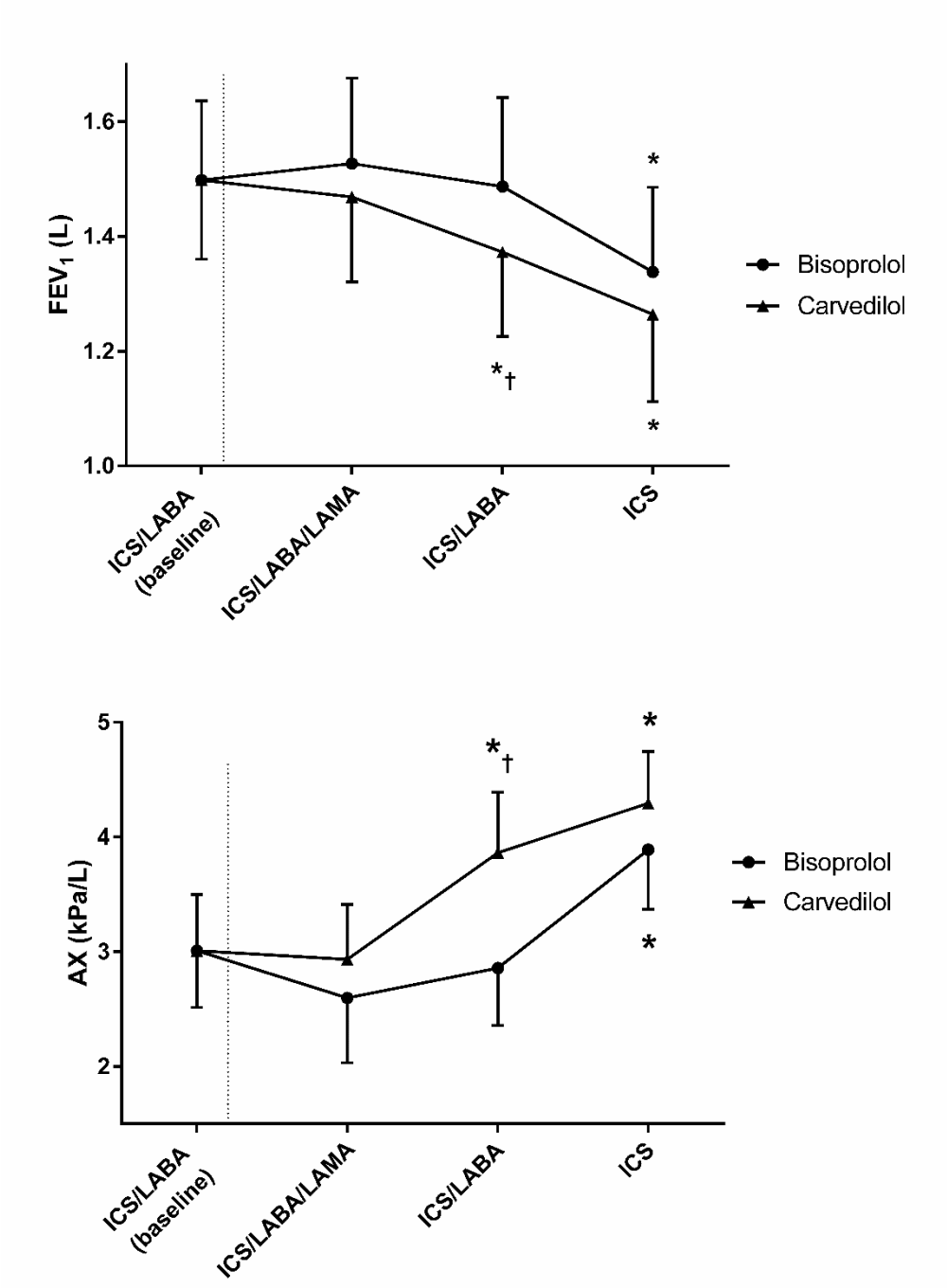


Figure 10.

Effects of bisoprolol and carvedilol on expiratory lung volume (FEV_1) (top) and compliance (as reactance area: AX) (bottom), stratified according to sequential inhaled therapy. Asterisk denotes significant difference within each beta-blocker

from baseline (P<0.05), Cross denotes significant difference between beta-blockers (P<0.05).

Exercise (6-minute walk test: 6MWT):

Resting and exercise nadir oxygen saturations were not altered from baseline by any combination of beta blocker or inhaled therapy (Table 7). Comparing 6MWT at baseline and each treatment arm, there were significant reductions in bis/ISC/LABA/LAMA (25m) and bis/ICS (26m) both of which are considered to be of no clinical significance, as the changes were less than the minimally important difference of 30m (202) (Table 7). Modified Borg scale for dyspnoea and fatigue was greater after exercise, but this was less than the minimally important difference of 1.0 unit (34), there were no significant differences between beta-blockers (Table 7). Both beta-blockers produced a similar degree of heart rate reduction compared to baseline for pre and post exercise in the order of 20 beats per minute, irrespective of concomitant inhaled therapy (Table 7 and figure 11). Serum potassium levels were unchanged.

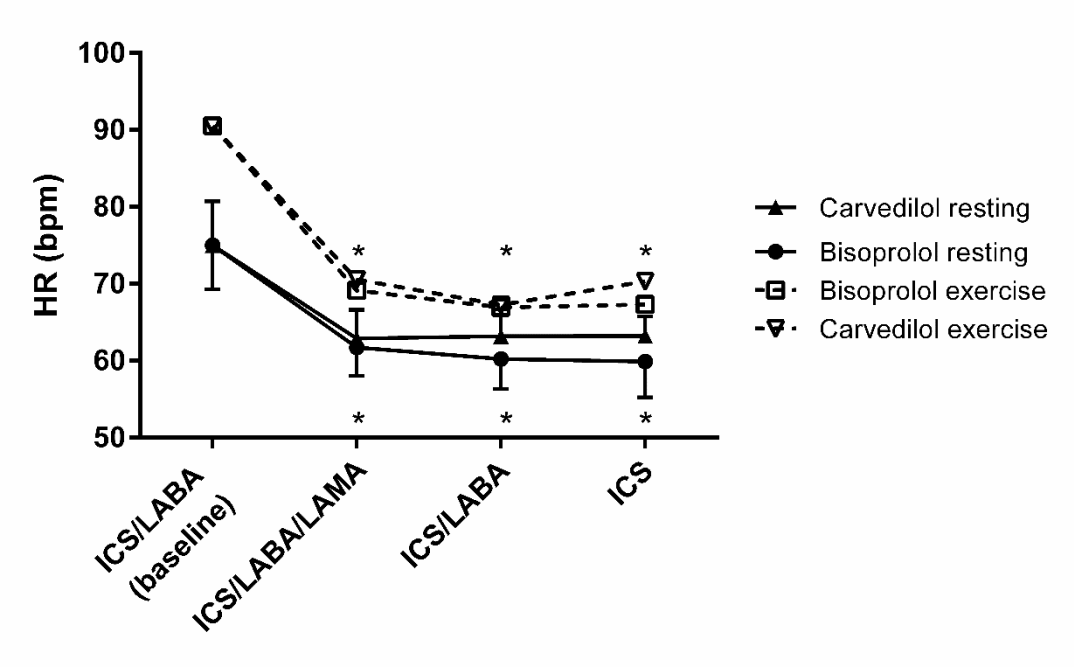


Figure 11.

Resting and post exercise heart rate on sequential inhaled therapy. Asterisk represents significant (P<0.05) vs baseline for both carvedilol and bisoprolol at each step of inhaled therapy. No between group differences observed.

Health status and Dyspnoea index:

For health status as SGRQ (Table 7) there were no significant changes from baseline and no differences between beta-blockers, with reference to a minimal important difference of 4 units(34).

The mean transition dyspnoea index (TDI) values were not significantly different between beta-blockers on any of the inhaled treatments. Values for both beta-blockers were lower on ICS vs ICS/LABA (Table 7), while for bisoprolol values were

also lower on ICS/LABA vs ICS/LABA/LAMA. Changes in TDI were less than the minimal important difference of 1.0 unit(34).

Table 7

	Pre beta-blocker	ICS/ LABA/LAMA Bisoprolol	ICS/ LABA/LAMA Carvedilol	ICS/ LABA Bisoprolol	ICS/ LABA Carvedilol	ICS Bisoprolol	ICS Carvedilol
Resting Sp02 (%)	96 (96-97)	96 (65-97)	96 (95-97)	96 (93-96)	96 (95-97)	96 (95-97)	96 (95-97)
Exercise Sp02 (%)	94 (91-96)	93 (90-96)	93 (90-95)	93 (90-95)	94 (91-96)	93 (90-95)	93 (90-95)
Resting HR (bpm)	75 (69-82)	62 (58-66)*	63 (59-67)*	60 (56-64)*	64 (60-68)*	60 (55-65)*	63 (61-66)*
Exercise HR (bpm)	91 (83-98)	69 (64-75)*	71 (67-74)*	67 (62-72)*	67 (57-77)*	67 (62-73)*	70 (67-74)*
Resting Systolic BP (mmHg)	135 (129-142)	129 (120-138)	127 (119-135)	127 (118-136)	128 (121-138)	127 (119-134)*	132 (122-142)
Exercise Systolic BP (mmHg)	151 (137-166)	151 (135-167)	152 (137-166)	149 (132-164)	145 (133-157)	145 (133-157)	151 (138-163)
Resting Diastolic BP (mmHg)	80 (76-84)	77 (71-83)	75 (70-79)*	72 (67-77)	76 (70-81)	73 (68-78)*	78 (72-83)
Exercise Diastolic BP (mmHg)	86 (82-91)	83 (76-89)	86 (81-91)	81 (75-87)*	81 (75-87)	83 (77-89)	87 (82-91)
Walk Distance (m)	495 (449-542)	470 (420-520)*	489 (449-542)	486 (435-536)	484 (422-567)	469 (422-515)*	474 (416-532)
SGRQ	33 (24-42)	34 (24, 44)	36 (27-45)	33 (24-42)	34 (25-43)	36 (28-44)	36 (26-45)
TDI (BDI)	6.7 (5.5-7.8)	0.83 (-0.24, 1.69)#	0.22 (-0.82, 1.27)	-0.33 (-1.31, 0.65)‡	0.22 (-0.69, 1.13)‡	-1.1 (-2.01, -0.21)	-0.83 (-1.92, 0.25)

Pooled baseline values (i.e. pre beta-blocker) are shown while taking ICS/LABA after run-in. There were no significant differences between beta blockers, *P<0.05 vs baseline. #P<0.05 ICS/LABA/LAMA vs ICS/LABA, ‡P<0.05 ICS/LABA vs ICS for either beta-blocker

Discussion

The results of the present study revealed some important new findings regarding beta-blockers and their interaction with long acting bronchodilators in patients with moderate to severe COPD.

In terms of pulmonary function values, non-selective beta blockers when administered to patients on ICS/LABA lead to worsening of FEV_1 and increase in AX (lung stiffness). This effect is not seen with the cardioselective beta blocker bisoprolol. The removal of the LABA leads to significant reductions in FEV_1 with both cardioselective and non-selective beta blockers. The addition of LAMA to ICS/LABA adds little to patients receiving bisoprolol but obliterates the reduction in FEV_1 and increase in AX seen in the carvedilol group, thus providing bronchoprotection. The increased AX may correlate to increased gas trapping and hyperinflation; in a previous study in COPD (204) it has been demonstrated that AX correlates most strongly with GOLD COPD disease severity, compared to the resistance IOS outcome measures such as R5, R20 and R5-R20.

Normally stimulation of inhibitory pre-junctional beta-2 receptors acts as a brake to post junctional cholinergic transmission (153). The presence of beta-2 receptor blockade would increase acetylcholine release which stimulates post junctional M3 receptors to produce constriction of airway smooth muscle. Hence, the speculation that tiotropium conferred protection against bronchoconstriction due to beta-2 receptor blockade with carvedilol. The same bronchoprotective effect of tiotropium with ICS has also been reported in asthma patients taking propranolol (155).

When the LAMA was stopped and patients subsequently continued ICS/LABA, the protective effect of tiotropium became evident as worsening of pulmonary function with carvedilol but not bisoprolol. A significant difference between beta-blockers was seen for spirometry (FEV_1, FVC) and reactance IOS indices (AX, X5) while taking ICS/LABA, which for FEV_1 exceeded the MCID of 100ml In this case, carvedilol negated the inhibitory effect of formoterol on pre-junctional beta2 receptors but without any accompanying post junctional M3 receptor antagonism. Post junctional beta-2 receptor stimulation by formoterol which normally produces bronchodilation, was also antagonised by carvedilol. However, pulmonary function was no worse with bisoprolol due to a negligible degree of beta-2 blockade associated with the 5mg dose due to its 14/1 β 1/2 selectivity ratio (193, 205).

When patients then stopped LABA and continued ICS alone, there was as expected further decline in pulmonary function with both beta-blockers due to the absence of formoterol induced bronchodilation as well as antagonism of circulating adrenaline. The clinical implication is that patients with COPD requiring beta-blockers should be prescribed concomitant LAMA to protect against potential bronchoconstriction, especially if using a non-selective drug. As selective beta-blockers exhibit dose related beta-2 receptor antagonism(206, 207) the concomitant use of LAMA may also be advocated when using at higher doses of bisoprolol (205).The likelihood is that most patients with COPD will already be taking a LAMA as current guidelines recommend the use of LAMA across GOLD stages 2-4, as they reduce exacerbations and improve quality of life (208). Moreover, in a retrospective study of

2853 COPD patients over 4·7 years, adding tiotropium to ICS/LABA was found to reduce respiratory and cardiovascular mortality (122).

Our results showed that both beta-blockers exhibited a comparable degree of heart rate reduction at both rest and after exercise, which in turn infers that bisoprolol 5mg qd and carvedilol 12·5 bid exhibited a similar degree of cardiac beta-1 blockade with a mean fall in the order of 20 beats per minute - a clinically meaningful response(209). We chose these pragmatic doses, for our COPD patients, as we considered that higher doses might not be tolerated in terms of symptomatic bradycardia and hypotension in a cohort of susceptible elderly patients (200). The observation of lowering of SBP and DBP with bisoprolol but not carvedilol was perhaps surprising given that carvedilol produces peripheral vasodilatation due to alpha-1 receptor blockade. However, at the same time the greater beta-2 receptor antagonism with carvedilol would attenuate vasodilatation due to stimulation of peripheral arterial beta-2 receptors(209).

As our patients did not have heart failure, we are unable to draw any meaningful conclusions with regards to the relative efficacy of these beta-blockers on cardiac function. In this regard it is conceivable that haemodynamic responses to beta-blockade might have been altered in heart failure patients. Although none of our patients were being treated for heart failure, we acknowledge that an echocardiogram would be required to diagnose cases of covert heart failure. For example, in a comparison of carvedilol and bisoprolol in 883 elderly patients with

heart failure over 12 weeks, both drugs produced comparable reductions in blood pressure and improvements in ejection fraction (200).

Jabbour et al also reported a significantly lower heart rate on carvedilol than bisoprolol amounting to 4 beats/min, whereas in our study we observed no such difference in chronotropic reduction. This discrepancy between the two studies may be explained by the presence of heart failure where the negative chronotropic effect of carvedilol might be enhanced due to a relatively higher proportion of cardiac beta-2 receptors. Our patients had a mean N-terminal pro-BNP level of 308 pg/ml. This has been described as the grey zone in COPD patients where they may be pulmonary arterial pressure overload and right ventricular wall stress (210).

The duration of treatment in each inhaler arm was of sufficient duration to achieve steady state effects in terms of potential pharmacodynamic interactions on both airway beta-2 and M3 receptors. One could argue that the step-down phase from ICS/LABA to ICS alone is not clinically relevant as the latter are not licensed in COPD. However, this allowed us to dissect out the effect of LABA by comparing ICS alone to prior ICS/LABA.

One unique aspect of our study was the use of IOS which is more sensitive than spirometry in detecting subtle changes in lung physiology. Unlike spirometry which measures lung volumes and flow, IOS measures lung resistance (R) and compliance (X) and is also able to differentiate between central and peripheral airways by looking at their frequency dependence, with lower frequencies reflecting changes in smaller airways.(126) Our study was powered on total airway resistance (R5) which did not

detect any significant effects of beta-blockade. Furthermore, there was no significant change in central (R20) or peripheral airway resistance (R5-R20). In contrast, there was a significant change in AX which reflects peripheral airways compliance, as the area under the reactance curve between 5Hz and the resonant frequency. This showed reduced lung compliance (as higher reactance: AX) for carvedilol versus bisoprolol in conjunction with either ICS/LABA or ICS alone.

The relative sensitivity of spirometry and IOS be a 9% versus 28% change in FEV_1 and AX respectively for ICS/LABA with carvedilol as change from baseline. The present findings comparing the relative effects of carvedilol on spirometry and IOS in COPD are similar to those previously reported in asthma with propranolol.(201)

The significant differences observed between beta-blockers on pulmonary function were not associated with commensurate differences on Borg scale (dyspnoea or fatigue), health status (SGRQ) or transition dyspnoea score (TDI). Bisoprolol with ICS significantly lowered 6MW distance compared to baseline by 26 metres, while the biggest difference between beta-blockers was 19 metres, both of which are less than the minimal important difference of 30m (202).

In summary, bisoprolol was better tolerated than carvedilol on pulmonary function at doses which produced equivalent cardiac beta-1 blockade. Worsening of pulmonary function with carvedilol was mitigated by concomitant inhaled tiotropium with formoterol, but not formoterol alone.

Further prospective long-term studies are warranted to compare bisoprolol and carvedilol in patients with heart failure and COPD to look at their relative effects on exacerbations and mortality.

Commentary:

It is disappointing this study did not meet its primary outcome and it is hard to ascertain the reasons why. This study proved difficult to recruit into, having been started by my predecessor Dr William Anderson (MD). In fact, the study was only finished a full year after my tenure at the SCRR, the last two patients having been completed by Dr Chris Kuo my successor. The main issue was the nature of recruiting patients with COPD. Of 45 patients screened 20 failed at the outset (prior to any randomisation). The reasons for this interestingly were not related to FEV_1 with only n=2 patients failing due to an FEV_1 <30% predicted. The most numerous reasons for screen failing was having a HR or BP less than the acceptable entry cut offs (HR<60, n= 4; SBP<110, n=2) prior to exposure to any beta blocker. Moreover, if a patient had to be withdrawn (n=7) it would take between 14-16 weeks to replace them with another completed patient, excluding time to find a participant. We cast a very wide net with the study data having multiple cardiopulmonary secondary endpoints. Overall, the cardiovascular effects were unsurprising and certainly not novel (reduction in HR and BP). That left us with the pulmonary tolerability, which did have novel data in terms of selective vs non- selective beta blockers and the bronchoprotection conferred by LAMA. The role of IOS in COPD remains mostly within the realms of research, and so has yet to gain mainstream traction. Mainly this

paper was criticised by the top tier respiratory journals for its small “n” size of 18 (which is the largest in this thesis). It was presented at poster discussion sessions at BTS and ERS, but only received a dozen citations since publication. It was the most complex and painstaking of all the data analysis conducted, but I had hoped for a slightly higher impact with its results.

Conclusion:

Trial	Primary Outcome:	Key Secondary Outcome:
NCT02039011 Non-smoking asthma ICS/LABA vs ICS/LABA/LAMA	No significant difference in AHR when LAMA added to ICS/LABA	No significant differences between spiro or IOS measurements when LAMA added to ICS/LABA
NCT02682862 Smoking asthma ICS/LABA vs ICS/LABA/LAMA	Significant but small difference in trough R5 at end of treatment when LAMA added to ICS/LABA	Significantly lower AX and ACQ score when LAMA added to ICS/LABA
NCT01656005 COPD ICS/LABA vs ICS/LABA/LAMA	No significant difference in R5 when LAMA added to ICS/LABA	Withdrawal of LAMA results in significantly lower FEV_1, FVC, and AX when patients are on a non-selective beta blocker

Table 8.

Summary findings of RCTs within thesis.

These data represent new insight into the role of dual bronchodilator in obstructive airway disease and complement what is already known about such therapy in asthma and COPD. With regards to non-smoking asthma, it was known that in severe disease, add on tiotropium to ICS/LABA reduced exacerbations, but the mechanism for this was not clear. It was uncertain whether this was related to airway calibre alone, as improvements in FEV_1 in the two largest RCTs (59, 60) were below

the MCID for asthma(61) . These data suggest that add on tiotropium to ICS/LABA in mild to moderate asthma did not have any impact on airway geometry (measured with impulse oscillometry) or airway calibre. Nor was there any potential attenuation of AHR. As tiotropium is only licensed for poorly controlled (severe) asthma, these data would suggest no clear benefit in mild to moderate asthma. The small sample size and single RCT nature of this study limit this being a confirmatory statement, potentially powering the study on AHR again, but including patients with more than mild AHR or patients with asthma not perfectly controlled would make for a more robust study design. The failure of AHR as the primary outcome meant that for the follow-on smoking asthma study, R5 was set as the primary outcome.

With regards to smokers with asthma, their inherent corticosteroid resistance has clinical interplay in their asthma therapy. It is perhaps unsurprising moreover that the enhanced bronchoconstrictor stimuli brought on by smoking is responsive to add on LAMA to ICS/LABA. Strictly speaking the BTS/GINA asthma guideline groups treat smoking asthma patients in the same way as non-smoking asthma, i.e. no special consideration is made. LAMA is utilised late on into asthma therapy once ICS/LABA has been increased to high dose and other secondary controllers (such as LTRA) have been considered. The lack of specific recommendations for smokers may be because this group is often underrepresented and poorly studied. In our smoking asthma study, we demonstrated significant improvements in trough airway resistance (R5), which may represent a time when the airways are most vulnerable. There were also significant improvements in objective asthma symptom questionnaires (ACQ) which were not present in the ICS/LABA arm. These data

would suggest that smokers with asthma, who often have poorer control than their non-smoking counterparts, would stand to benefit most from add on LAMA to ICS/LABA. Tiotropium remains, at the time of writing, the only licensed LAMA for asthma and, as mentioned previously, it is reserved for patients with poorly controlled asthma who still exacerbate despite ICS/LABA. The main downside here is that this was not a comparative study of smokers vs non-smokers, and again it was a single centre trial which had a primary outcome of essentially research interest. It is uncertain whether a minute reduction in airway resistance really means anything, but the ACQ data was promising. As there is no MCID in IOS interpretation of the R5 signal is speculative.

With regards to COPD, the benefits of dual bronchodilation with LABA/LAMA are well studied, in terms of reduction in exacerbations (211), lung function, quality of life, and mortality (122, 123) versus ICS/LABA. The recently developed closed combined triple inhaler therapy (ICS/LABA/LAMA) has demonstrated superiority against LABA/LAMA, but only in terms of a modest reduction in further exacerbations (35, 212). The predominant initial drug of choice in COPD is bronchodilator therapy (single, then escalating to dual based on symptoms), with add on ICS only being considered in exacerbating patients, as per the license of the two commercially available ICS/LABA/LAMA inhalers: Trimbow (beclometasone/formoterol/ glycopyrronium) and Trelegy Ellipta (fluticasone furoate/ umeclidinium/vilanterol). Our data demonstrates that dual bronchodilation provides bronchoprotection from non-selective beta-blockade in terms of airway reactance (AX) and airway calibre (FEV_1). This protection against bronchoconstrictor stimulus may in turn provide

protection against exacerbation. The study had the secondary benefit of demonstrating that beta blockers (both cardioselective and non-selective) were well tolerated as therapeutic doses in patients with moderate to severe COPD. This data is clinically useful to prescribers and is perhaps the only study of the three which had categorically clear results. The utility of LAMA became clearer when it was withdrawn and patients on non-selective beta blockers experienced deterioration in pulmonary function both in terms of IOS and spirometry values. The main problem of the study was the recruitment, for patients it was essentially very labour intensive with multiple long visits to collect a surfeit of secondary outcomes, most of which were insignificant. A repeat study using the modern single device ICS/LABA/LAMA inhalers and removing secondary outcomes like 6MWT, would have potentially made for a more streamlined study, or at least attracted more participants. A larger prospective trial is underway (Beta Blockers in COPD) assessing whether bisoprolol reduces exacerbations in patients with COPD. Our study did not look at exacerbations due its comparatively short duration. Any exacerbation study in COPD is conventionally over one year, including the two recent COPD ICS/LABA/LAMA licensing studies(212, 213).

Since the publication of the three studies within this thesis, two single device ICS/LABA/LAMA therapies have been introduced as the mainstay of COPD management as described above, but they are also more recently licensed for the management of severe asthma in the form of Trimbow (180) and Trelegy (214). Both these studies demonstrate that the add on LAMA had small improvements in FEV_1 vs ICS/LABA; 110mls for Trelegy (214) and 57mls for Trimbow (180), but these

improvements were below the FEV_1 MCID of 230mls in asthma (34). Neither of these studies assessed any degree of AHR and only Trimbow demonstrated a significant (15%) reduction in exacerbations, Trelegy failed to show any reduction in asthma exacerbations. Considering these patients were poorly controlled more severe asthmatics, a trivial improvement in FEV_1 seems inconsequential. Our AHR studies remain unique in the sense that most large trials do not look at this end point, leaving room for further smaller mechanistic trials in this group of patients. Most importantly both these studies excluded current smokers who, as ever, remain an understudied group and underrepresented in asthma. Whilst they are an important disease group to understand and treat the paradigm of recruitment remains. A smoker with asthma who is interested enough in their health to participate in RCTs but remains committed to smoking is a rare breed. We had trouble recruiting that study.

The management of obstructive airway disease should focus on the underlying treatable trait, this is now a mainstream concept (3). In non-smokers with asthma this may be eosinophilic or type 1 airway inflammation, in smoking asthma it may be a combined picture of type 1 and type 2 (one of which may predominate), finally in COPD it may primarily be non-eosinophilic or type 2 airway inflammation.

Our data would suggest that LAMA is a useful add on to ICS/LABA in smoking asthma, but less relevant in non-smoking asthma outside the realm of severe disease. In COPD we demonstrated that LAMA is exceptionally useful in preventing bronchoconstriction from beta blockers. Therefore, its role is perhaps best in those

who express a more type 2 airway disease trait, in simpler words more bronchitic, less asthmatic.

Understanding what drives underlying airway disease will allow us to move from a simple additive strategy (and increasing drug burden) to a targeted treatment strategy, thereby ensuring the right treatment to the right lungs.

Publications:

1: Lipworth BJ, Jabbal S. What can we learn about COPD from impulse oscillometry? Respir Med. 2018 Jun;139:106-109. doi: 10.1016/j.rmed.2018.05.004. Epub 2018 May 16.
2: Lipworth B, Kuo CR, Jabbal S. Current appraisal of single inhaler triple therapy in COPD. Int J Chron Obstruct Pulmon Dis. 2018 Sep 28;13:3003-3009. doi:10.2147/COPD.S177333.
3: Lipworth B, Jabbal S. Debate on long-acting β agonists for asthma: they think it's all over. Lancet Respir Med. 2017 Mar;5(3):e14-e15. doi:10.1016/S2213-2600(17)30053-X.
4: Lipworth BJ, Jabbal S. Un-diagnosing persistent adult asthma. Eur Respir J. 2017 Nov 2;50(5):1701433. doi: 10.1183/13993003.01433-2017.
5: Lipworth B, Jabbal S, Kuo CR. Anti-interleukin 13 for asthma: stick or twist? Lancet Respir Med. 2018 Sep;6(9):e46-e47. doi: 10.1016/S2213-2600(18)30275-3. Epub 2018 Aug 2.
6: Kuo CR, Jabbal S, Anderson W, Lipworth BJ. Pragmatic evaluation of inhaled corticosteroid particle size formulations on asthma control. Clin Exp Allergy. 2019 Oct;49(10):1321-1327. doi: 10.1111/cea.13453. Epub 2019 Aug 6.
7: Kuo CR, Jabbal S, Lipworth B. I Say IOS You Say AOS: Comparative Bias in Respiratory Impedance Measurements. Lung. 2019 Aug;197(4):473-481. doi: 10.1007/s00408-019-00247-y. Epub 2019 Jul 4.
8: Lipworth B, Kuo CR, Jabbal S. Inhaled triple therapy in chronic obstructive pulmonary disease. Lancet. 2018 Sep 29;392(10153):1112-1113. doi: 10.1016/S0140-6736(18)31795-1.
9: Lipworth BJ, Jabbal S. Reappraisal of the clinical effect of mepolizumab. Lancet Respir Med. 2017 Jun;5(6):e20. doi: 10.1016/S2213-2600(17)30183-2. Epub 2017 May 26.
10: Lipworth B, Kuo C, Jabbal S. Adrenal suppression with inhaled corticosteroids: the seed and the soil. Lancet Respir Med. 2018 Jun;6(6):e19.

doi: 10.1016/S2213-2600(18)30148-6.
11: Jabbal S, Kuo CR, Lipworth B. Randomized controlled trial of triple versus dual inhaler therapy on small airways in smoking asthmatics. Clin Exp Allergy. 2020 Oct;50(10):1140-1147. doi: 10.1111/cea.13702. Epub 2020 Jul 27.
12: Singh D, van den Berg F, Leaker B, Corradi M, Jabbal S, Collarini S, Mongelli V, Santoro L, Piccinno A, Biondaro S, Lipworth B. Comparison of the effect of beclometasone/formoterol in asthma patients after methacholine-induced bronchoconstriction: A noninferiority study using metered dose vs. dry powder inhaler. Br J Clin Pharmacol. 2019 Apr;85(4):729-736. doi: 10.1111/bcp.13847. Epub 2019 Feb 3.
13: Lipworth BJ, Jabbal S. FULFIL an Unmet Need in Chronic Obstructive Pulmonary Disease. Am J Respir Crit Care Med. 2017 Oct 15;196(8):1082. doi: 10.1164/rccm.201704-0728LE.
14: Jabbal S, Lipworth BJ. Tolerability of Bisoprolol on Domiciliary Spirometry in COPD. Lung. 2018 Feb;196(1):11-14. doi: 10.1007/s00408-017-0061-y. Epub 2017. Oct 14.
15: Lipworth B, Jabbal S. A pragmatic approach to simplify inhaler therapy for COPD. Lancet Respir Med. 2017 Sep;5(9):679-681. doi: 10.1016/S2213-2600(17)30264-3. Epub 2017 Jul 7
16: Lipworth B, Jabbal S. Impact of Spacers on Therapeutic Ratio with Inhaled Corticosteroids. J Allergy Clin Immunol Pract. 2017 Jul-Aug;5(4):1163. doi: 10.1016/j.jaip.2017.03.011.
17: Jabbal S, Lipworth BJ. Does the asthma visual analog scale relate to the Asthma Control Questionnaire? Ann Allergy Asthma Immunol. 2018 May;120(5):533-535. doi: 10.1016/j.anai.2017.12.026. Epub 2018 Mar 21.
18: Lipworth B, Jabbal S, Manoharan A, Anderson W, Short P. Inhaled corticosteroid dose-response on blood eosinophils in asthma. Lancet Respir Med. 2016 Jan;4(1):e1. doi: 10.1016/S2213-2600(15)00504-4.
19: Kuo CR, Jabbal S, Lipworth B. Is small airways dysfunction related to asthma control and type 2 inflammation? Ann Allergy Asthma Immunol. 2018

Nov;121(5):631-632. doi: 10.1016/j.anai.2018.08.009. Epub 2018 Aug 19.
20: Jabbal S, Lipworth BJ. Blood eosinophils: The forgotten man of inhaled steroid dose titration. Clin Exp Allergy. 2018 Jan;48(1):93-95. doi: 10.1111/cea.13057. Epub 2017 Dec 1.
21: Jabbal S, Anderson W, Short P, Morrison A, Manoharan A, Lipworth BJ. Cardiopulmonary interactions with beta-blockers and inhaled therapy in COPD. QJM. 2017 Dec 1;110(12):785-792. doi: 10.1093/qjmed/hcx155.
22: Anderson WJ, Short PM, Jabbal S, Lipworth BJ. Inhaled corticosteroid dose response in asthma: Should we measure inflammation? Ann Allergy Asthma Immunol. 2017 Feb;118(2):179-185. doi: 10.1016/j.anai.2016.11.018. Epub 2017 Jan 3.
23: Lipworth BJ, Jabbal S. Drug-device interaction for systemic effects of fluticasone in patients with asthma. Ann Allergy Asthma Immunol. 2017 Aug;119(2):194. doi: 10.1016/j.anai.2017.01.023.
24: Jabbal S, Poli G, Lipworth B. Does size really matter?: Relationship of particle size to lung deposition and exhaled fraction. J Allergy Clin Immunol. 2017 Jun;139(6):2013-2014.e1. doi: 10.1016/j.jaci.2016.11.036. Epub 2017 Jan 10.
25: Lipworth B, Jabbal S. Of mice and men-the curious tale of β blockers in asthma. Lancet Respir Med. 2016 Feb;4(2):89-91. doi: 10.1016/S2213-2600(16)00011-4.
26: Jabbal S, Manoharan A, Lipworth BJ. Bronchoprotective tolerance with indacaterol is not modified by concomitant tiotropium in persistent asthma. Clin Exp Allergy. 2017 Oct;47(10):1239-1245. doi: 10.1111/cea.12972. Epub 2017 Aug 1.
27: Jabbal S, Manoharan A, Lipworth J, Lipworth B. Utility of impulse oscillometry in patients with moderate to severe persistent asthma. J Allergy Clin Immunol. 2016 Aug;138(2):601-3. doi: 10.1016/j.jaci.2015.12.1336. Epub 2016 Mar 23.
28: Jabbal S, Manoharan A, Anderson W, Lipworth J, Lipworth B. Real-life effect

of long-acting β2 agonist withdrawal in patients with controlled step 3 asthma. Ann Allergy Asthma Immunol. 2016 Oct;117(4):430-431. doi: 10.1016/j.anai.2016.07.022. Epub 2016 Aug 9.

29: Jabbal S, Lipworth BJ. Sensitivity of Lung Resistance and Compliance to Beta-Blocker Induced Bronchoconstriction and Long Acting Beta-Agonist Withdrawal in COPD. Lung. 2018 Feb;196(1):15-18. doi: 10.1007/s00408-017-0079-1. Epub 2017 Dec 20.

30: Jabbal S, Manoharan A, Lipworth J, Anderson W, Short P, Lipworth B. Is Gly16Arg β2 Receptor Polymorphism Related to Impulse Oscillometry in a Real-Life Asthma Clinic Setting? Lung. 2016 Apr;194(2):267-71. doi: 10.1007/s00408-016-9848-5. Epub 2016 Feb 15.

31: Lipworth B, RuiWen Kuo C, Jabbal S, Chan R. Inhaled triple therapy and airway hyperresponsiveness in persistent asthma. Ann Allergy Asthma Immunol. 2021 May;126(5):597-598. doi: 10.1016/j.anai.2021.01.032. Epub 2021 Feb 3.

Congress:

- European Respiratory Society: Annual Congress, London, September 2016:
    - Late breaking oral abstract: Dr Sunny Jabbal; “Real life impact of long acting beta-2-agonist withdrawal in controlled step 3 asthma patients”

- European Respiratory Society: Annual Congress, Milan, September 2017:
    - Late breaking oral abstract: Dr Sunny Jabbal: “Blood eosinophils in inhaled steroid dose titration”
    - Poster discussion 1: Bronchoprotective tolerance with indacaterol is not modified by concomitant tiotropium in persistent asthma
    - Poster discussion 2: Cardiopulmonary interactions with beta-blockers and inhaled therapy in COPD

- British Thoracic Society: Winter Meeting: London, December 2016. Moderated Poster presentation:
    - P 239 Effects of tiotropium on asthma exacerbations are not explained by airway hyperresponsiveness, exhaled breath nitric oxide or airway geometry.
- British Thoracic Society: Winter Meeting: London, December 2017. Moderated Poster presentation:
    - P230 Does the global asthma visual analogue scale relate to the asthma control questionnaire?
    - P277 Bisoprolol blunts domiciliary FEV1 in COPD patients taking concomitant dual or triple inhaler therapy.

Table 9.

Publications in order of date, related to work at the SCRR. This thesis represents only a small proportion of work and research output generated during my time at the SCRR. All studies within this work are published in peer reviewed journals, but for the purposes of the thesis appear in fuller forms, rather than the more stripped-down nature of a research publication.

References:

1. Postma DS, Weiss ST, van den Berge M, Kerstjens HA, Koppelman GH. Revisiting the Dutch hypothesis. The Journal of allergy and clinical immunology. 2015;136(3):521-9.
2. Fletcher CM, Pride NB. Definitions of emphysema, chronic bronchitis, asthma, and airflow obstruction: 25 years on from the Ciba symposium. Thorax. 1984;39(2):81-5.
3. Pavord ID, Beasley R, Agusti A, Anderson GP, Bel E, Brusselle G, et al. After asthma: redefining airways diseases. Lancet. 2017.
4. VM M, SA H, K G, ES H, GB M, M H, et al. Treatable traits can be identified in a severe asthma registry and predict future exacerbations. Respirology (Carlton, Vic). 2019;24(1).
5. SE W. Asthma phenotypes: the evolution from clinical to molecular approaches. Nature medicine. 2012;18(5).
6. 2017 GINA Report: Global Strategy for Asthma Management and Prevention 2017 [Available from: http://ginasthma.org/2017-gina-report-global-strategy-for-asthma-management-and-prevention/.
7. From the Global Strategy for the Diagnosis, Management and Prevention of COPD, Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2017. Available from: http://goldcopd.org. 2017.
8. Martinez FD, Vercelli D. Asthma. Lancet (London, England). 2013;382(9901):1360-72.
9. Bax HJ, King's College London RDoCMB, King's College London, Guy's Campus, New Hunt"s House, London, SE11UL, United Kingdom, Heather.bax@kcl.ac.uk, Keeble AH, King's College London RDoCMB, King's College London, Guy's Campus, New Hunt"s House, London, SE11UL, United Kingdom, Anthony.keeble@kcl.ac.uk, et al. Cytokinergic IgE Action in Mast Cell Activation. Frontiers in Immunology. 2012;0.
10. Chibana K, Trudeau JB, Mustovich AT, Hu H, Zhao J, Balzar S, et al. IL-13 induced increases in nitrite levels are primarily driven by increases in inducible nitric oxide synthase as compared with effects on arginases in human primary bronchial epithelial cells. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2008;38(6):936-46.
11. Ricciardolo FL. Multiple roles of nitric oxide in the airways. Thorax. 2003;58(2):175-82.
12. Prado CM, Leick-Maldonado EA, Yano L, Leme AS, Capelozzi VL, Martins MA, et al. Effects of nitric oxide synthases in chronic allergic airway inflammation and remodeling. American journal of respiratory cell and molecular biology. 2006;35(4):457-65.
13. Menzies D, Nair A, Lipworth BJ. Portable exhaled nitric oxide measurement: Comparison with the "gold standard" technique. Chest. 2007;131(2):410-4.
14. Barnes ML, Menzies D, Fardon TC, Burns P, Wilson AM, Lipworth BJ. Combined mediator blockade or topical steroid for treating the unified allergic airway. Allergy. 2007;62(1):73-80.
15. Anderson WJ, Lipworth BJ. Relationship of mannitol challenge to methacholine challenge and inflammatory markers in persistent asthmatics receiving inhaled corticosteroids. Lung. 2012;190(5):513-21.
16. Anderson WJ, Short PM, Williamson PA, Lipworth BJ. Inhaled corticosteroid dose response using domiciliary exhaled nitric oxide in persistent asthma: the FENOtype trial. Chest. 2012;142(6):1553-61.

17. Jabbal S, Lipworth BJ. Blood eosinophils: The forgotten man of inhaled steroid dose titration. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2018;48(1):93-5.
18. A B, AM S, AO B, HO Q, RK G, IA S. Association Between FeNO, Total Blood IgE, Peripheral Blood Eosinophil and Inflammatory Cytokines in Partly Controlled Asthma. Journal of asthma and allergy. 2020;13.
19. Bardsley G, Daley-Yates P, Baines A, Kempsford R, Williams M, Mallon T, et al. Anti-inflammatory duration of action of fluticasone furoate/vilanterol trifenatate in asthma: a cross-over randomised controlled trial. Respiratory research. 2018;19(1):133.
20. Heaney LG, Busby J, Bradding P, Chaudhuri R, Mansur AH, Niven R, et al. Remotely Monitored Therapy and Nitric Oxide Suppression Identifies Nonadherence in Severe Asthma. American journal of respiratory and critical care medicine. 2019;199(4):454-64.
21. Roth M, Johnson PR, Rudiger JJ, King GG, Ge Q, Burgess JK, et al. Interaction between glucocorticoids and beta2 agonists on bronchial airway smooth muscle cells through synchronised cellular signalling. Lancet (London, England). 2002;360(9342):1293-9.
22. Kaur M, Chivers JE, Giembycz MA, Newton R. Long-acting beta2-adrenoceptor agonists synergistically enhance glucocorticoid-dependent transcription in human airway epithelial and smooth muscle cells. Molecular pharmacology. 2008;73(1):203-14.
23. Sindi A, Todd DC, Nair P. Antiinflammatory effects of long-acting beta2-agonists in patients with asthma: a systematic review and metaanalysis. Chest. 2009;136(1):145-54.
24. Johnston SL, Edwards MR. Mechanisms of adverse effects of β-agonists in asthma. 2009.
25. Kistemaker LE, Hiemstra PS, Bos IS, Bouwman S, van den Berge M, Hylkema MN, et al. Tiotropium attenuates IL-13-induced goblet cell metaplasia of human airway epithelial cells. Thorax. 2015;70(7):668-76.
26. Casale TB, Bateman ED, Vandewalker M, Virchow JC, Schmidt H, Engel M, et al. Tiotropium Respimat Add-on Is Efficacious in Symptomatic Asthma, Independent of T2 Phenotype. J Allergy Clin Immunol Pract. 2018;6(3):923-35.e9.
27. Fardon T, Haggart K, Lee DK, Lipworth BJ. A proof of concept study to evaluate stepping down the dose of fluticasone in combination with salmeterol and tiotropium in severe persistent asthma. Respiratory medicine. 2007;101(6):1218-28.
28. K W, P T, Y F, Y W, C L. Assessment of second-line treatments for patients with uncontrolled moderate asthma. International journal of clinical and experimental medicine. 2015;8(10).
29. Silva GE, Sherrill DL, Guerra S, Barbee RA. Asthma as a risk factor for COPD in a longitudinal study. Chest. 2004;126(1):59-65.
30. Lundback B, Lindberg A, Lindstrom M, Ronmark E, Jonsson AC, Jonsson E, et al. Not 15 but 50% of smokers develop COPD?--Report from the Obstructive Lung Disease in Northern Sweden Studies. Respiratory medicine. 2003;97(2):115-22.
31. Barnes PJ. How corticosteroids control inflammation: Quintiles Prize Lecture 2005. British journal of pharmacology. 1482006. p. 245-54.
32. Keatings VM, Jatakanon A, Worsdell YM, Barnes PJ. Effects of inhaled and oral glucocorticoids on inflammatory indices in asthma and COPD. American journal of respiratory and critical care medicine. 1997;155(2):542-8.
33. Brightling CE, McKenna S, Hargadon B, Birring S, Green R, Siva R, et al. Sputum eosinophilia and the short term response to inhaled mometasone in chronic obstructive pulmonary disease. 2005.

34. Jones PW, Beeh KM, Chapman KR, Decramer M, Mahler DA, Wedzicha JA. Minimal clinically important differences in pharmacological trials. Am J Respir Crit Care Med. 2014;189(3):250-5.
35. A P, J V, L F, M C, H P, G C, et al. Extrafine Inhaled Triple Therapy Versus Dual Bronchodilator Therapy in Chronic Obstructive Pulmonary Disease (TRIBUTE): A Double-Blind, Parallel Group, Randomised Controlled Trial. Lancet (London, England). 2018;391(10125).
36. Barnes PJ. Asthma-COPD Overlap. Chest. 2016;149(1):7-8.
37. DMG H, GJ C, A P, D S, A A, FJ M, et al. Global Initiative for the Diagnosis, Management, and Prevention of Chronic Obstructive Lung Disease. The 2020 GOLD Science Committee Report on COVID-19 and Chronic Obstructive Pulmonary Disease. American journal of respiratory and critical care medicine. 2021;203(1).
38. Tu X, Donovan C, Kim RY, Wark PAB, Horvat JC, Hansbro PM. Asthma-COPD overlap: current understanding and the utility of experimental models. 2021.
39. Christenson SA, Steiling K, van den Berge M, Hijazi K, Hiemstra PS, Postma DS, et al. Asthma-COPD overlap. Clinical relevance of genomic signatures of type 2 inflammation in chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine. 2015;191(7):758-66.
40. Rogliani P, Ora J, Puxeddu E, Cazzola M. Airflow obstruction: is it asthma or is it COPD? International journal of chronic obstructive pulmonary disease. 112016. p. 3007-13.
41. Pera T, Penn RB. Crosstalk between beta-2-adrenoceptor and muscarinic acetylcholine receptors in the airway. Current opinion in pharmacology. 2014;16:72-81.
42. Proskocil BJ, Fryer AD. β2-Agonist and Anticholinergic Drugs in the Treatment of Lung Disease. http://dxdoiorg/101513/pats200504-038SR. 2012.
43. Moulton BC, Fryer AD. Muscarinic receptor antagonists, from folklore to pharmacology; finding drugs that actually work in asthma and COPD. British journal of pharmacology. 2011;163(1):44-52.
44. D W, S J, X Z. Crosstalk between pathways enhances the controllability of signalling networks. IET systems biology. 2016;10(1).
45. Asthma GIf. 2016 GINA Report: Global Strategy for Asthma Management and Prevention - Global Initiative for Asthma - GINA. 2016.
46. Haney S, Hancox RJ. Rapid onset of tolerance to beta-agonist bronchodilation. Respiratory medicine. 2005;99(5):566-71.
47. Haney S, Hancox RJ. Tolerance to bronchodilation during treatment with long-acting beta-agonists, a randomised controlled trial. Respiratory research. 62005. p. 107.
48. Nishikawa M, Mak JC, Barnes PJ. Effect of short- and long-acting beta 2-adrenoceptor agonists on pulmonary beta 2-adrenoceptor expression in human lung. European journal of pharmacology. 1996;318(1):123-9.
49. Grove A, Lipworth BJ. Bronchodilator subsensitivity to salbutamol after twice daily salmeterol in asthmatic patients. Lancet (London, England). 1995;346(8969):201-6.
50. Hui KK, Conolly ME, Tashkin DP. Reversal of human lymphocyte beta-adrenoceptor desensitization by glucocorticoids. Clinical pharmacology and therapeutics. 1982;32(5):566-71.
51. Aziz I, Lipworth BJ. A bolus of inhaled budesonide rapidly reverses airway subsensitivity and beta2-adrenoceptor down-regulation after regular inhaled formoterol. Chest. 1999;115(3):623-8.
52. Aziz I, McFarlane LC, Lipworth BJ. Concomitant inhaled corticosteroid resensitises cardiac beta2-adrenoceptors in the presence of long-acting beta2-agonist therapy. European journal of clinical pharmacology. 1998;54(5):377-81.

53. Tan KS, McFarlane LC, Lipworth BJ. Concomitant administration of low-dose prednisolone protects against in vivo beta2-adrenoceptor subsensitivity induced by regular formoterol. Chest. 1998;113(1):34-41.
54. Tan KS, Grove A, McLean A, Gnosspelius Y, Hall IP, Lipworth BJ. Systemic corticosteriod rapidly reverses bronchodilator subsensitivity induced by formoterol in asthmatic patients. American journal of respiratory and critical care medicine. 1997;156(1):28-35.
55. Lazarus SC, Boushey HA, Fahy JV, Chinchilli VM, Lemanske RF, Jr., Sorkness CA, et al. Long-acting beta2-agonist monotherapy vs continued therapy with inhaled corticosteroids in patients with persistent asthma: a randomized controlled trial. Jama. 2001;285(20):2583-93.
56. Ducharme FM, Ni Chroinin M, Greenstone I, Lasserson TJ. Addition of long-acting beta2-agonists to inhaled corticosteroids versus same dose inhaled corticosteroids for chronic asthma in adults and children. The Cochrane database of systematic reviews. 2010(5):Cd005535.
57. Peters SP, Bleecker ER, Canonica GW, Park YB, Ramirez R, Hollis S, et al. Serious Asthma Events with Budesonide plus Formoterol vs. Budesonide Alone. N Engl J Med. 2016;375(9):850-60.
58. B D, GA S, KL R, TJ W, R H. Tiotropium (Spiriva): mechanistical considerations and clinical profile in obstructive lung disease. Life sciences. 1999;64(6-7).
59. Kerstjens HA, Engel M, Dahl R, Paggiaro P, Beck E, Vandewalker M, et al. Tiotropium in asthma poorly controlled with standard combination therapy. N Engl J Med. 2012;367(13):1198-207.
60. Kerstjens HA, Disse B, Schroder-Babo W, Bantje TA, Gahlemann M, Sigmund R, et al. Tiotropium improves lung function in patients with severe uncontrolled asthma: a randomized controlled trial. J Allergy Clin Immunol. 2011;128(2):308-14.
61. Santanello NC, Zhang J, Seidenberg B, Reiss TF, Barber BL. What are minimal important changes for asthma measures in a clinical trial? Eur Respir J. 1999;14(1):23-7.
62. Lipworth B, Manoharan A, Short P. Tiotropium in asthma. N Engl J Med. 2012;367(26):2552; author reply 3.
63. Juniper EF, Guyatt GH, Cox FM, Ferrie PJ, King DR. Development and validation of the Mini Asthma Quality of Life Questionnaire. The European respiratory journal. 1999;14(1):32-8.
64. Schatz M, Zeiger RS, Yang SJ, Chen W, Crawford W, Sajjan S, et al. The relationship of asthma impairment determined by psychometric tools to future asthma exacerbations. Chest. 2012;141(1):66-72.
65. Ohta S, Oda N, Yokoe T, Tanaka A, Yamamoto Y, Watanabe Y, et al. Effect of tiotropium bromide on airway inflammation and remodelling in a mouse model of asthma. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2010;40(8):1266-75.
66. Buels K, Jacoby D, Fryer A. Non-bronchodilating mechanisms of tiotropium prevent airway hyperreactivity in a guinea-pig model of allergic asthma. British journal of pharmacology. 2012;165(5):1501-14.
67. Cockcroft DW, Killian DN, Mellon JJ, Hargreave FE. Bronchial reactivity to inhaled histamine: a method and clinical survey. Clinical allergy. 1977;7(3):235-43.
68. Laprise C, Laviolette M, Boutet M, Boulet LP. Asymptomatic airway hyperresponsiveness: relationships with airway inflammation and remodelling. Eur Respir J. 1999;14(1):63-73.
69. Postma DS, Kerstjens HA. Characteristics of airway hyperresponsiveness in asthma and chronic obstructive pulmonary disease. American journal of respiratory and critical care medicine. 1998;158(5 Pt 3):S187-92.
70. Schwartz N, Grossman A, Levy Y, Schwarz Y. Correlation between eosinophil count and methacholine challenge test in asymptomatic subjects. J Asthma. 2012;49(4):336-41.

71. Jatakanon A, Lim S, Kharitonov SA, Chung KF, Barnes PJ. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. Thorax. 1998;53(2):91-5.
72. Juniper EF, Frith PA, Hargreave FE. Airway responsiveness to histamine and methacholine: relationship to minimum treatment to control symptoms of asthma. Thorax. 1981;36(8):575-9.
73. Peat JK, Woolcock AJ, Cullen K. Rate of decline of lung function in subjects with asthma. European journal of respiratory diseases. 1987;70(3):171-9.
74. Lipworth BJ, Jabbal S. Un-diagnosing persistent adult asthma. The European respiratory journal. 2017;50(5).
75. Tashkin DP, Altose MD, Bleecker ER, Connett JE, Kanner RE, Lee WW, et al. The lung health study: airway responsiveness to inhaled methacholine in smokers with mild to moderate airflow limitation. The Lung Health Study Research Group. The American review of respiratory disease. 1992;145(2 Pt 1):301-10.
76. Crapo RO, Casaburi R, Coates AL, Enright PL, Hankinson JL, Irvin CG, et al. Guidelines for methacholine and exercise challenge testing-1999. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. American journal of respiratory and critical care medicine. 2000;161(1):309-29.
77. Brand PL, Postma DS, Kerstjens HA, Koeter GH. Relationship of airway hyperresponsiveness to respiratory symptoms and diurnal peak flow variation in patients with obstructive lung disease. The Dutch CNSLD Study Group. The American review of respiratory disease. 1991;143(5 Pt 1):916-21.
78. Rutgers SR, Koeter GH, van der Mark TW, Postma DS. Short-term treatment with budesonide does not improve hyperresponsiveness to adenosine 5'-monophosphate in COPD. American journal of respiratory and critical care medicine. 1998;157(3 Pt 1):880-6.
79. Weir DC, Bale GA, Bright P, Sherwood Burge P. A double-blind placebo-controlled study of the effect of inhaled beclomethasone dipropionate for 2 years in patients with nonasthmatic chronic obstructive pulmonary disease. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 1999;29 Suppl 2:125-8.
80. Cockcroft DW. Direct challenge tests: Airway hyperresponsiveness in asthma: its measurement and clinical significance. Chest. 2010;138(2 Suppl):18s-24s.
81. Joos GF, O'Connor B, on behalf of the Task Force C-aotTFRGFJC, B. O'Connor (Co-Chairman), S.D. Anderson, F. Chung, D.W. Cockcroft, B. Dahlén, G. DiMaria, A. Foresi, F.E. Hargreave, S.T. Holgate, M. Inman, J. Lötvall, H. Magnussen, R. Polosa, D.S. Postma, J. Riedler. Indirect airway challenges. 2003.
82. Joos GF, O'Connor B, Anderson SD, Chung F, Cockcroft DW, Dahlen B, et al. Indirect airway challenges. The European respiratory journal. 2003;21(6):1050-68.
83. Van Schoor J, Joos GF, Pauwels RA. Indirect bronchial hyperresponsiveness in asthma: mechanisms, pharmacology and implications for clinical research. The European respiratory journal. 2000;16(3):514-33.
84. A S, C P, SF T, V B. Diagnostic properties of inhaled mannitol in the diagnosis of asthma: a population study. The Journal of allergy and clinical immunology. 2009;124(5).
85. Pauwels R, Joos G, Van der Straeten M. Bronchial hyperresponsiveness is not bronchial hyperresponsiveness is not bronchial asthma. Clinical allergy. 1988;18(4):317-21.
86. Van Den Berge M, Meijer RJ, Kerstjens HA, de Reus DM, Koeter GH, Kauffman HF, et al. PC(20) adenosine 5'-monophosphate is more closely associated with airway inflammation in asthma than PC(20) methacholine. American journal of respiratory and critical care medicine. 2001;163(7):1546-50.

87. Benckhuijsen J, van den Bos JW, van Velzen E, de Bruijn R, Aalbers R. Differences in the effect of allergen avoidance on bronchial hyperresponsiveness as measured by methacholine, adenosine 5'-monophosphate, and exercise in asthmatic children. Pediatric pulmonology. 1996;22(3):147-53.
88. Currie GP, Haggart K, Lee DK, Fowler SJ, Wilson AM, Brannan JD, et al. Effects of mediator antagonism on mannitol and adenosine monophosphate challenges. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2003;33(6):783-8.
89. Wood LG, Powell H, Gibson PG. Mannitol challenge for assessment of airway responsiveness, airway inflammation and inflammatory phenotype in asthma. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2010;40(2):232-41.
90. Porsbjerg C, Lund TK, Pedersen L, Backer V. Inflammatory subtypes in asthma are related to airway hyperresponsiveness to mannitol and exhaled NO. J Asthma. 2009;46(6):606-12.
91. Attanasi M, Consilvio NP, Rapino D, Nicola MD, Scaparrotta A, Cingolani A, et al. Bronchial hyperresponsiveness to mannitol, airway inflammation and Asthma Control Test in atopic asthmatic children. Arch Med Sci. 122016. p. 137-44.
92. Lipworth BJ, Short PM, Williamson PA, Clearie KL, Fardon TC, Jackson CM. A randomized primary care trial of steroid titration against mannitol in persistent asthma: STAMINA trial. Chest. 2012;141(3):607-15.
93. Sont JK, Willems LN, Bel EH, van Krieken JH, Vandenbroucke JP, Sterk PJ. Clinical control and histopathologic outcome of asthma when using airway hyperresponsiveness as an additional guide to long-term treatment. The AMPUL Study Group. Am J Respir Crit Care Med. 1999;159(4 Pt 1):1043-51.
94. Blanc FX, Macedo P, Hew M, Chung KF. Capsaicin cough sensitivity in smokers with and without airflow obstruction. Respiratory medicine. 2009;103(5):786-90.
95. Mullen JB, Wiggs BR, Wright JL, Hogg JC, Pare PD. Nonspecific airway reactivity in cigarette smokers. Relationship to airway pathology and baseline lung function. The American review of respiratory disease. 1986;133(1):120-5.
96. Gerrard JW, Cockcroft DW, Mink JT, Cotton DJ, Poonawala R, Dosman JA. Increased nonspecific bronchial reactivity in cigarette smokers with normal lung function. The American review of respiratory disease. 1980;122(4):577-81.
97. Piccillo G, Caponnetto P, Barton S, Russo C, Origlio A, Bonaccorsi A, et al. Changes in airway hyperresponsiveness following smoking cessation: comparisons between Mch and AMP. Respiratory medicine. 2008;102(2):256-65.
98. Jetmalani K, Thamrin C, Farah CS, Bertolin A, Chapman DG, Berend N, et al. Peripheral airway dysfunction and relationship with symptoms in smokers with preserved spirometry. Respirology (Carlton, Vic). 2018;23(5):512-8.
99. Bickel S, Popler J, Lesnick B, Eid N. Impulse Oscillometry: Interpretation and Practical Applications. CHEST Journal. 2015;146(3):841-7.
100. Jabbal S, Manoharan A, Lipworth J, Lipworth B. Utility of impulse oscillometry in patients with moderate to severe persistent asthma. The Journal of allergy and clinical immunology. 2016.
101. Chaudhuri R, McSharry C, McCoard A, Livingston E, Hothersall E, Spears M, et al. Role of symptoms and lung function in determining asthma control in smokers with asthma. Allergy. 2008;63(1):132-5.
102. Vozoris NT, Stanbrook MB. Smoking prevalence, behaviours, and cessation among individuals with COPD or asthma. Respir Med. 2011;105(3):477-84.

103. To T, Stanojevic S, Moores G, Gershon AS, Bateman ED, Cruz AA, et al. Global asthma prevalence in adults: findings from the cross-sectional world health survey. BMC Public Health. 2012;12:204.
104. Eisner MD, Yelin EH, Katz PP, Shiboski SC, Henke J, Blanc PD. Predictors of cigarette smoking and smoking cessation among adults with asthma. Am J Public Health. 2000;90(8):1307-11.
105. Chalmers GW, Macleod KJ, Little SA, Thomson LJ, McSharry CP, Thomson NC. Influence of cigarette smoking on inhaled corticosteroid treatment in mild asthma. Thorax. 2002;57(3):226-30.
106. Lazarus SC, Chinchilli VM, Rollings NJ, Boushey HA, Cherniack R, Craig TJ, et al. Smoking Affects Response to Inhaled Corticosteroids or Leukotriene Receptor Antagonists in Asthma. Am J Respir Crit Care Med. 2012.
107. Lange P, Parner J, Vestbo J, Schnohr P, Jensen G. A 15-year follow-up study of ventilatory function in adults with asthma. N Engl J Med. 1998;339(17):1194-200.
108. Polosa R, Thomson NC. Smoking and asthma: dangerous liaisons. Eur Respir J. 2013;41(3):716-26.
109. Peters SP, Kunselman SJ, Icitovic N, Moore WC, Pascual R, Ameredes BT, et al. Tiotropium Bromide Step-Up Therapy for Adults with Uncontrolled Asthma. N Engl J Med. 2010;363(18):1715-26.
110. Beeh KM, Moroni-Zentgraf P, Ablinger O, Hollaenderova Z, Unseld A, Engel M, et al. Tiotropium Respimat® in asthma: a double-blind, randomised, dose-ranging study in adult patients with moderate asthma. Respiratory research. 152014. p. 61.
111. NJ G, E C, MS S. Cholinergic bronchomotor tone in COPD. Estimates of its amount in comparison with that in normal subjects. Chest. 1989;96(5).
112. Ahmad Z, Singh SK. Relative and additional bronchodilator response of salbutamol and ipratropium in smoker and nonsmoker asthmatics. J Asthma. 2010;47(3):340-3.
113. Yoshida M, Kaneko Y, Ishimatsu A, Komori M, Iwanaga T, Inoue H. Effects of tiotropium on lung function in current smokers and never smokers with bronchial asthma. Pulmonary pharmacology & therapeutics. 2017;42:7-12.
114. Mio T, Romberger DJ, Thompson AB, Robbins RA, Heires A, Rennard SI. Cigarette smoke induces interleukin-8 release from human bronchial epithelial cells. Am J Respir Crit Care Med. 2012.
115. Costa L, Roth M, Miglino N, Keglowich L, Zhong J, Lardinois D, et al. Tiotropium sustains the anti-inflammatory action of olodaterol via the cyclic AMP pathway. Pulm Pharmacol Ther. 2014;27(1):29-37.
116. Short PM, Lipworth SI, Elder DH, Schembri S, Lipworth BJ. Effect of beta blockers in treatment of chronic obstructive pulmonary disease: a retrospective cohort study. BMJ (Clinical research ed). 2011;342:d2549.
117. Agusti A, Calverley PM, Celli B, Coxson HO, Edwards LD, Lomas DA, et al. Characterisation of COPD heterogeneity in the ECLIPSE cohort. Respiratory research. 2010;11:122.
118. Hospers JJ, Postma DS, Rijcken B, Weiss ST, Schouten JP. Histamine airway hyper-responsiveness and mortality from chronic obstructive pulmonary disease: a cohort study. Lancet (London, England). 2000;356(9238):1313-7.
119. Lipworth B, Skinner D, Devereux G, Thomas V, Ling Zhi Jie J, Martin J, et al. Underuse of beta-blockers in heart failure and chronic obstructive pulmonary disease. Heart (British Cardiac Society). 2016;102(23):1909-14.

120. McDonough JE, Yuan R, Suzuki M, Seyednejad N, Elliott WM, Sanchez PG, et al. Small-airway obstruction and emphysema in chronic obstructive pulmonary disease. N Engl J Med. 2011;365(17):1567-75.
121. Day K, kg5n14@soton.ac.uk, Faculty of Medicine UoS, Southampton, NIHR Southampton Biomedical Research Centre UHS, Southampton, Ostridge K, Faculty of Medicine UoS, Southampton, et al. Interrelationships Among Small Airways Dysfunction, Neutrophilic Inflammation, and Exacerbation Frequency in COPD. CHEST. 2021;159(4):1391-9.
122. Short PM, Williamson PA, Elder DHJ, Lipworth SIW, Schembri S, Lipworth BJ. The impact of tiotropium on mortality and exacerbations when added to inhaled corticosteroids and long-acting beta-agonist therapy in COPD. Chest. 2012;141(1):81-6.
123. Tashkin DP, Celli B, Senn S, Burkhart D, Kesten S, Menjoge S, et al. A 4-year trial of tiotropium in chronic obstructive pulmonary disease. N Engl J Med. 2008;359(15):1543-54.
124. Bonini M, Usmani OS. The role of the small airways in the pathophysiology of asthma and chronic obstructive pulmonary disease. Therapeutic advances in respiratory disease. 2015;9(6):281-93.
125. Yanai M, Sekizawa K, Ohrui T, Sasaki H, Takishima T. Site of airway obstruction in pulmonary disease: direct measurement of intrabronchial pressure. Journal of applied physiology (Bethesda, Md : 1985). 1992;72(3):1016-23.
126. Lipworth B, Manoharan A, Anderson W. Unlocking the quiet zone: the small airway asthma phenotype. The Lancet Respiratory medicine. 2014;2(6):497-506.
127. Perez T, Chanez P, Dusser D, Devillier P. Small airway impairment in moderate to severe asthmatics without significant proximal airway obstruction. Respiratory medicine. 2013;107(11):1667-74.
128. Manoharan A, Anderson WJ, Lipworth J, Ibrahim I, Lipworth BJ. Small airway dysfunction is associated with poorer asthma control. The European respiratory journal. 44. England2014. p. 1353-5.
129. Hogg JC, Chu F, Utokaparch S, Woods R, Elliott WM, Buzatu L, et al. The nature of small-airway obstruction in chronic obstructive pulmonary disease. N Engl J Med. 2004;350(26):2645-53.
130. Borrill ZL, Houghton CM, Woodcock AA, Vestbo J, Singh D. Measuring bronchodilation in COPD clinical trials. Br J Clin Pharmacol. 2005;59(4):379-84.
131. Dubois AB, Brody AW, Lewis DH, Burgess BF, Jr. Oscillation mechanics of lungs and chest in man. Journal of applied physiology. 1956;8(6):587-94.
132. Criee CP, Sorichter S, Smith HJ, Kardos P, Merget R, Heise D, et al. Body plethysmography--its principles and clinical use. Respiratory medicine. 2011;105(7):959-71.
133. Tomalak W, Radlinski J, Pawlik J, Latawier W, Pogorzelski A. Impulse oscillometry vs. body plethysmography in assessing respiratory resistance in children. Pediatric pulmonology. 2006;41(1):50-4.
134. Naji N, Keung E, Kane J, Watson RM, Killian KJ, Gauvreau GM. Comparison of changes in lung function measured by plethymography and IOS after bronchoprovocation. Respiratory medicine. 2013;107(4):503-10.
135. Crim C, Celli B, Edwards LD, Wouters E, Coxson HO, Tal-Singer R, et al. Respiratory system impedance with impulse oscillometry in healthy and COPD subjects: ECLIPSE baseline results. Respiratory medicine. 2011;105(7):1069-78.
136. Quint JK, Herrett E, Bhaskaran K, Timmis A, Hemingway H, Wedzicha JA, et al. Effect of beta blockers on mortality after myocardial infarction in adults with COPD: population based cohort study of UK electronic healthcare records. BMJ. 2013;347:f6650.

137. Egred M, Shaw S, Mohammad B, Waitt P, Rodrigues E. Under-use of beta-blockers in patients with ischaemic heart disease and concomitant chronic obstructive pulmonary disease. QJM : monthly journal of the Association of Physicians. 2005;98(7):493-7.
138. Chen J, Radford MJ, Wang Y, Marciniak TA, Krumholz HM. Effectiveness of beta-blocker therapy after acute myocardial infarction in elderly patients with chronic obstructive pulmonary disease or asthma. J Am Coll Cardiol. 2001;37(7):1950-6.
139. Standardization of Spirometry, 1994 Update. American Thoracic Society. Am J Respir Crit Care Med. 1995;152(3):1107-36.
140. Brashier B, Salvi S. Measuring lung function using sound waves: role of the forced oscillation technique and impulse oscillometry system. Breathe (Sheffield, England). 112015. p. 57-65.
141. Anderson SD, Brannan J, Spring J, Spalding N, Rodwell LT, Chan K, et al. A new method for bronchial-provocation testing in asthmatic subjects using a dry powder of mannitol. American journal of respiratory and critical care medicine. 1997;156(3 Pt 1):758-65.
142. Alving K, Anolik R, Crater G, LaForce CF, Rickard K. Validation of a New Portable Exhaled Nitric Oxide Analyzer, NIOX VERO®: Randomized Studies in Asthma. Pulmonary Therapy. 2017;3(1):207-18.
143. Spahn JD, Malka J, Szefler SJ. Current application of exhaled nitric oxide in clinical practice. The Journal of allergy and clinical immunology. 2016;138(5):1296-8.
144. Kougias M, Vardavas CI, Anagnostopoulos N, Matsunaga Y, Tzwrtzi A, Lymberi M, et al. The acute effect of cigarette smoking on the respiratory function and FENO production among young smokers. Experimental lung research. 2013;39(8):359-64.
145. Chinoy B, Yee E, Bahna SL. Skin testing versus radioallergosorbent testing for indoor allergens. Clinical and molecular allergy : CMA. 2005;3(1):4.
146. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. The European respiratory journal. 1999;14(4):902-7.
147. Juniper EF, Bousquet J, Abetz L, Bateman ED. Identifying 'well-controlled' and 'not well-controlled' asthma using the Asthma Control Questionnaire. Respiratory medicine. 2006;100(4):616-21.
148. Jones PW, Quirk FH, Baveystock CM. The St George's Respiratory Questionnaire. Respiratory medicine. 1991;85 Suppl B:25-31; discussion 3-7.
149. Mahler DA, Weinberg DH, Wells CK, Feinstein AR. The measurement of dyspnea. Contents, interobserver agreement, and physiologic correlates of two new clinical indexes. Chest. 1984;85(6):751-8.
150. ATS statement: guidelines for the six-minute walk test. American journal of respiratory and critical care medicine. 2002;166(1):111-7.
151. Borg GA. Psychophysical bases of perceived exertion. Medicine and science in sports and exercise. 1982;14(5):377-81.
152. Fryer AD, Jacoby DB. Muscarinic receptors and control of airway smooth muscle. American journal of respiratory and critical care medicine. 1998;158(5 Pt 3):S154-60.
153. Lipworth BJ. Emerging role of long acting muscarinic antagonists for asthma. Br J Clin Pharmacol. 2014;77(1):55-62.
154. Boterman M, Smits SR, Meurs H, Zaagsma J. Protein kinase C potentiates homologous desensitization of the beta2-adrenoceptor in bovine tracheal smooth muscle. Eur J Pharmacol. 2006;529(1-3):151-6.

155. Short PM, Anderson WJ, Williamson PA, Lipworth BJ. Effects of intravenous and oral beta-blockade in persistent asthmatics controlled on inhaled corticosteroids. Heart (British Cardiac Society). 2014;100(3):219-23.
156. Kistemaker LE, Oenema TA, Meurs H, Gosens R. Regulation of airway inflammation and remodeling by muscarinic receptors: perspectives on anticholinergic therapy in asthma and COPD. Life sciences. 2012;91(21-22):1126-33.
157. Gosens R, Bos IS, Zaagsma J, Meurs H. Protective effects of tiotropium bromide in the progression of airway smooth muscle remodeling. American journal of respiratory and critical care medicine. 2005;171(10):1096-102.
158. O'Connor BJ, Towse LJ, Barnes PJ. Prolonged effect of tiotropium bromide on methacholine-induced bronchoconstriction in asthma. American journal of respiratory and critical care medicine. 1996;154(4 Pt 1):876-80.
159. Vogelmeier C, Ramos-Barbon D, Jack D, Piggott S, Owen R, Higgins M, et al. Indacaterol provides 24-hour bronchodilation in COPD: a placebo-controlled blinded comparison with tiotropium. Respiratory research. 2010;11:135.
160. Currie GP, Haggart K, Brannan JD, Lee DK, Anderson SD, Lipworth BJ. Relationship between airway hyperresponsiveness to mannitol and adenosine monophosphate. Allergy. 2003;58(8):762-6.
161. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J. 2005;26(2):319-38.
162. American Thoracic S, European Respiratory S. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. American journal of respiratory and critical care medicine. 2005;171(8):912-30.
163. Lipworth B, Tan S, Devlin M, Aiken T, Baker R, Hendrick D. Effects of treatment with formoterol on bronchoprotection against methacholine. The American journal of medicine. 1998;104(5):431-8.
164. Aziz I, Tan KS, Hall IP, Devlin MM, Lipworth BJ. Subsensitivity to bronchoprotection against adenosine monophosphate challenge following regular once-daily formoterol. European Respiratory Journal. 1998;12(3):580-4.
165. Tan KS, Grove A, McLean A, Gnosspelius Y, Hall IP, Lipworth BJ. Systemic corticosteriod rapidly reverses bronchodilator subsensitivity induced by formoterol in asthmatic patients. American Journal of Respiratory and Critical Care Medicine. 1997;156(1):28-35.
166. Lipworth BJ, Aziz I. A high dose of albuterol does not overcome bronchoprotective subsensitivity in asthmatic subjects receiving regular salmeterol or formoterol. The Journal of allergy and clinical immunology. 1999;103(1 Pt 1):88-92.
167. Battram C, Charlton SJ, Cuenoud B, Dowling MR, Fairhurst RA, Farr D, et al. In vitro and in vivo pharmacological characterization of 5-[(R)-2-(5,6-diethyl-indan-2-ylamino)-1-hydroxy-ethyl]-8-hydroxy-1H-quinolin-2-o ne (indacaterol), a novel inhaled beta(2) adrenoceptor agonist with a 24-h duration of action. J Pharmacol Exp Ther. 2006;317(2):762-70.
168. Naline E, Trifilieff A, Fairhurst RA, Advenier C, Molimard M. Effect of indacaterol, a novel long-acting beta2-agonist, on isolated human bronchi. Eur Respir J. 2007;29(3):575-81.
169. Newnham DM, McDevitt DG, Lipworth BJ. Bronchodilator subsensitivity after chronic dosing with eformoterol in patients with asthma. American Journal of Medicine. 1994;97(1):29-37.
170. Newnham DM, Grove A, McDevitt DG, Lipworth BJ. Subsensitivity of bronchodilator and systemic beta 2 adrenoceptor responses after regular twice daily treatment with eformoterol dry powder in asthmatic patients. Thorax. 1995;50(5):497-504.

171. Aziz I, Lipworth BJ. A bolus of inhaled budesonide rapidly reverses airway subsensitivity and ?2-adrenoceptor down-regulation after regular inhaled formoterol. Chest. 1999;115(3):623-8.
172. Britton J, Hanley SP, Garrett HV, Hadfield JW, Tattersfield AE. Dose related effects of salbutamol and ipratropium bromide on airway calibre and reactivity in subjects with asthma. Thorax. 1988;43(4):300-5.
173. Koskela HO, Hyvarinen L, Brannan JD, Chan HK, Anderson SD. Sensitivity and validity of three bronchial provocation tests to demonstrate the effect of inhaled corticosteroids in asthma. Chest. 2003;124(4):1341-9.
174. TS H, JD L, G J, GL H, KH C, DA K, et al. ERS technical standard on bronchial challenge testing: pathophysiology and methodology of indirect airway challenge testing. The European respiratory journal. 2018;52(5).
175. Short PM, Williamson PA, Lipworth BJ. Sensitivity of impulse oscillometry and spirometry in beta-blocker induced bronchoconstriction and beta-agonist bronchodilatation in asthma. Ann Allergy Asthma Immunol. 2012;109(6):412-5.
176. Nair A, Ward J, Lipworth BJ. Comparison of bronchodilator response in patients with asthma and healthy subjects using spirometry and oscillometry. Ann Allergy Asthma Immunol. 2011;107(4):317-22.
177. Crim C, Celli B, Edwards LD, Wouters E, Coxson HO, Tal-Singer R, et al. Respiratory system impedance with impulse oscillometry in healthy and COPD subjects: ECLIPSE baseline results. Respir Med. 2011;105(7):1069-78.
178. Niewoehner DE, Kleinerman J, Rice DB. Pathologic changes in the peripheral airways of young cigarette smokers. N Engl J Med. 1974;291(15):755-8.
179. Cosio MG, Hale KA, Niewoehner DE. Morphologic and morphometric effects of prolonged cigarette smoking on the small airways. Am Rev Respir Dis. 1980;122(2):265-21.
180. Virchow JC, Kuna P, Paggiaro P, Papi A, Singh D, Corre S, et al. Single inhaler extrafine triple therapy in uncontrolled asthma (TRIMARAN and TRIGGER): two double-blind, parallel-group, randomised, controlled phase 3 trials. Lancet. 2019;394(10210):1737-49.
181. Mio T, Romberger DJ, Thompson AB, Robbins RA, Heires A, Rennard SI. Cigarette smoke induces interleukin-8 release from human bronchial epithelial cells. American journal of respiratory and critical care medicine. 1997;155(5):1770-6.
182. Kerstjens HA, Engel M, Dahl R, Paggiaro P, Beck E, Vandewalker M, et al. Tiotropium in Asthma Poorly Controlled with Standard Combination Therapy. N Engl J Med. 2012.
183. Jabbal S, Manoharan A, Lipworth BJ. Bronchoprotective tolerance with indacaterol is not modified by concomitant tiotropium in persistent asthma. Clin Exp Allergy. 2017;47(10):1239-45.
184. Cazzola M, Di Marco F, Santus P, Boveri B, Verga M, Matera MG, et al. The pharmacodynamic effects of single inhaled doses of formoterol, tiotropium and their combination in patients with COPD. Pulmonary pharmacology & therapeutics. 2004;17(1):35-9.
185. Lipworth B. Targeting the small airways asthma phenotype: if we can reach it, should we treat it? Ann Allergy Asthma Immunol. 2013;110(4):233-9.
186. Williamson PA, Clearie K, Menzies D, Vaidyanathan S, Lipworth BJ. Assessment of small-airways disease using alveolar nitric oxide and impulse oscillometry in asthma and COPD. Lung. 2011;189(2):121-9.
187. Jabbal S, Anderson W, Short P, Morrison A, Manoharan A, Lipworth BJ. Cardiopulmonary interactions with beta-blockers and inhaled therapy in COPD. Qjm. 2017.
188. Koumis T, Samuel S. Tiotropium bromide: a new long-acting bronchodilator for the treatment of chronic obstructive pulmonary disease. Clin Ther. 2005;27(4):377-92.

189. Beeh KM, Westerman J, Kirsten AM, Hebert J, Gronke L, Hamilton A, et al. The 24-h lung-function profile of once-daily tiotropium and olodaterol fixed-dose combination in chronic obstructive pulmonary disease. Pulm Pharmacol Ther. 2015;32:53-9.
190. Casaburi R, Briggs DD, Jr., Donohue JF, Serby CW, Menjoge SS, Witek TJ, Jr. The spirometric efficacy of once-daily dosing with tiotropium in stable COPD: a 13-week multicenter trial. The US Tiotropium Study Group. Chest. 2000;118(5):1294-302.
191. Postma DS, Rabe KF. The Asthma-COPD Overlap Syndrome. N Engl J Med. 2015;373(13):1241-9.
192. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. European journal of heart failure. 2016;18(8):891-975.
193. Baker JG. The selectivity of beta-adrenoceptor antagonists at the human beta1, beta2 and beta3 adrenoceptors. British journal of pharmacology. 2005;144(3):317-22.
194. Bristow MR, Ginsburg R, Umans V, Fowler M, Minobe W, Rasmussen R, et al. Beta 1- and beta 2-adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective beta 1-receptor down-regulation in heart failure. Circ Res. 1986;59(3):297-309.
195. Poole-Wilson PA, Swedberg K, Cleland JG, Di Lenarda A, Hanrath P, Komajda M, et al. Comparison of carvedilol and metoprolol on clinical outcomes in patients with chronic heart failure in the Carvedilol Or Metoprolol European Trial (COMET): randomised controlled trial. Lancet. 2003;362(9377):7-13.
196. McMurray JJ, Adamopoulos S, Anker SD, Auricchio A, Bohm M, Dickstein K, et al. ESC guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. Eur J Heart Fail. 2012;14(8):803-69.
197. Vestbo J, Hurd SS, Agusti AG, Jones PW, Vogelmeier C, Anzueto A, et al. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. American journal of respiratory and critical care medicine. 2013;187(4):347-65.
198. Du Q, Sun Y, Ding N, Lu L, Chen Y. Beta-blockers reduced the risk of mortality and exacerbation in patients with COPD: a meta-analysis of observational studies. PLoS One. 2014;9(11):e113048.
199. Lipworth B, Wedzicha J, Devereux G, Vestbo J, Dransfield MT. Beta-blockers in COPD: time for reappraisal. Eur Respir J. 2016.
200. Dungen HD, Apostolovic S, Inkrot S, Tahirovic E, Topper A, Mehrhof F, et al. Titration to target dose of bisoprolol vs. carvedilol in elderly patients with heart failure: the CIBIS-ELD trial. Eur J Heart Fail. 2011;13(6):670-80.
201. Short PM, Williamson PA, Lipworth BJ. Sensitivity of Impulse Oscillometry and Spirometry in the Assessment of Beta-Blocker Induced Bronchoconstriction and Beta-Agonist Bronchodilatation in Mild-to-Moderate Asthmatics. Thorax. 2011;66:A7-A.
202. Polkey MI, Spruit MA, Edwards LD, Watkins ML, Pinto-Plata V, Vestbo J, et al. Six-minute-walk test in chronic obstructive pulmonary disease: minimal clinically important difference for death or hospitalization. American journal of respiratory and critical care medicine. 2013;187(4):382-6.

203. JL J, R vK, J L, A B-G, J O-L, M S-B, et al. NT-proBNP testing for diagnosis and short-term prognosis in acute destabilized heart failure: an international pooled analysis of 1256 patients: the International Collaborative of NT-proBNP Study. European heart journal. 2006;27(3).
204. Wei X, Shi Z, Cui Y, Mi J, Ma Z, Ren J, et al. Impulse oscillometry system as an alternative diagnostic method for chronic obstructive pulmonary disease. Medicine (Baltimore). 2017;96(46):e8543-e.
205. Lipworth BJ, Irvine NA, McDevitt DG. A dose-ranging study to evaluate the beta 1-adrenoceptor selectivity of bisoprolol. European Journal of Clinical Pharmacology. 1991;40(2):135-9.
206. Lipworth BJ, Brown RA, McDevitt DG. Assessment of airways, tremor and chronotropic responses to inhaled salbutamol in the quantification of ?2-adrenoceptor blockade. Br J Clin Pharmacol. 1989;28(1):95-102.
207. Wheeldon NM, McDevitt DG, Lipworth BJ. Selectivity of antagonist and partial agonist activity of celiprolol in normal subjects. British Journal of Clinical Pharmacology. 1992;34(4):337-43.
208. Tashkin DP, Celli B, Senn S, Burkhart D, Kesten S, Menjoge S, et al. A 4-year trial of tiotropium in chronic obstructive pulmonary disease. N Engl J Med. 2008;359(15):1543-54.
209. Arnold JM, O'Connor PC, Riddell JG, Harron DW, Shanks RG, McDevitt DG. Effects of the beta 2-adrenoceptor antagonist ICI 118,551 on exercise tachycardia and isoprenaline-induced beta-adrenoceptor responses in man. Br J Clin Pharmacol. 1985;19(5):619-30.
210. Maisel A, Mueller C, Adams K, Jr., Anker SD, Aspromonte N, Cleland JG, et al. State of the art: using natriuretic peptide levels in clinical practice. European journal of heart failure. 2008;10(9):824-39.
211. Wedzicha JA, Banerji D, Chapman KR, Vestbo J, Roche N, Ayers RT, et al. Indacaterol-Glycopyrronium versus Salmeterol-Fluticasone for COPD. N Engl J Med. 2016.
212. DA L, F B, N B, J B, GJ C, NC D, et al. Once-Daily Single-Inhaler Triple Versus Dual Therapy in Patients With COPD. The New England journal of medicine. 2018;378(18).
213. D S, A P, M C, I P, I M, C F, et al. Single inhaler triple therapy versus inhaled corticosteroid plus long-acting β2-agonist therapy for chronic obstructive pulmonary disease (TRILOGY): a double-blind, parallel group, randomised controlled trial. Lancet (London, England). 2016;388(10048).
214. LA L, Z B, N B, LP B, D E, A F, et al. Efficacy and safety of once-daily single-inhaler triple therapy (FF/UMEC/VI) versus FF/VI in patients with inadequately controlled asthma (CAPTAIN): a double-blind, randomised, phase 3A trial. The Lancet Respiratory medicine. 2021;9(1).