Long-Acting Bronchodilators and Risk of Myocardial Infarction in Patients with Chronic Obstructive Pulmonary Disease Who are at High Risk for Cardiovascular Disease: a Quasi-Cohort Approach

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ABSTRACT

Objective: Long-acting bronchodilators are the mainstay of pharmacologic therapy for moderate to severe chronic obstructive pulmonary disease (COPD), yet the concern regarding their cardiovascular safety remains. This study aimed to evaluate whether use of long-acting bronchodilators increases the risk of acute myocardial infarction (MI) in patients with COPD who are at high risk for cardiovascular disease.

Methods: A new-user cohort of patients 55 years of age or greater who were prescribed at least one long-acting bronchodilator from September 2003 to August 2011 was identified using the Clinical Practice Research Datalink (CPRD) and followed from the first prescription up to a maximum of two years. The study cohort was further restricted to patients at high risk for cardiovascular disease at cohort entry. All cases of acute MI occurring during follow-up were identified and up to 5 quasi-cohort person-moments were selected at random. The association between current long-acting bronchodilator use and acute MI was estimated using a quasi-cohort approach, focusing on users of long-acting β2-agonists (LABA) and long-acting muscarinic antagonist (LAMA) together, LABA alone and LAMA alone.

Results: The cohort included 76,965 subjects, with 1,462 who had the outcome event of acute MI during more than 49.6 million person-days of follow-up (rate of acute MI: 10.8 per 1,000 person-years). The adjusted quasi-rate ratios of LABA and LAMA together, LABA alone and LAMA alone were 1.06 (95 % confidence interval [CI]: 0.82 to 1.37), 1.04 (95% CI: 0.85 to 1.27) and 0.91 (95% CI: 0.74 to 1.11), respectively, relative to no current use. The adjusted quasi-rate differences were 0.65 (95% CI: -2.15 to 3.37), 0.37 (95% CI: -1.88 to 2.45) and -0.99 (95% CI: -3.13 to 0.98) per 1,000 person-years for LABA and LAMA together, LABA alone and LAMA alone, respectively.

Conclusion: The use of LABA and LAMA, given alone or together, do not increase the risk of acute MI in patients with COPD at high risk for cardiovascular disease.

RÉSUMÉ

Objectif: Les bronchodilatateurs à action prolongée sont des traitements communs pour ceux souffrant de la maladie pulmonaire obstructive chronique (MPOC) modérée à sévère, mais l'inquiétude concernant leur sécurité cardiovasculaire demeure présente. Cette étude a comme but d'évaluer si les bronchodilatateurs à action prolongée augmentent les risques d'infarctus du myocarde (IDM) dans les patients souffrant de MPOC qui sont à haut risque de maladie cardiovasculaire.

Méthode: Une cohorte de patients âgés de 55 ans et plus, qui furent prescrit au moins un bronchodilatateur à action prolongée de septembre 2003 à août 2011, sont identifiés utilisant le Clinical Practice Research Datalink (CPRD) et suivis à partir de leur première prescription jusqu'à un maximum de deux ans. La cohorte était ensuite restreinte aux patients ayant un risque cardiovasculaire élevé. Tous les nouveaux cas d'IDM aigüe pendant la période de suivi sont identifiés et un maximum de 5 quasi-cohorte personne-moments témoins sont choisis au hasard. L'association entre l'utilisation présente de bronchodilatateurs à action prolongée et IDM aigü sont estimés utilisant une approche quasi-cohorte, examinant l'utilisation des combinaisons de bêta2-agoniste à longue durée d'action (BALA) et antagoniste muscarinique à longue durée d'action (AMLA), BALA seul et AMLA seul.

Résultats: La cohorte contient 76 965 sujets, avec 1 462 qui ont un d'IDM aigü pendant plus que 49,6 millions jours-personne de suivi (le taux de IDM aigü: 10.8 par 1 000 années-personne). Les quasi-rapport de taux ajustés de BALA et AMLA ensemble, BALA seul et AMLA seul sont 1.06 (95 % intervalle de confiance [IC]: 0.82 à 1.37), 1.04 (95 % IC: 0.85 à 1.27) et 0.91 (95 % IC: 0.74 à 1.11), respectivement, comparant à ceux qui ne les utilisent pas. Les quasi-taux différences ajustés sont 0.65 (95% IC: -2.15 à 3.37), 0.37 (95 % IC: -1.88 à 2.45) et -0.99 (95 % IC: -3.13 à 0.98) par 1 000 années-personne pour BALA et AMLA ensemble, BALA seul et AMLA seul, respectivement.

Conclusion: L'utilisation de BALA et AMLA, utilisés seul ou ensemble, n'augmente pas le risque d'IDM aigü chez les patients souffrant de MPOC qui sont à haut risque de maladie cardiovasculaire.

PREFACE

This thesis aims to evaluate whether use of long-acting bronchodilators increases the risk of acute MI in patients with COPD who are at high risk for cardiovascular disease.

The first chapter is an introduction to COPD. It presents the prevalence and economic burden of the disease, provides definitions, diagnostic criteria, natural history of COPD, and management of stable COPD. The second chapter provides an extensive literature review on the use of various bronchodilators for patients with COPD, and discusses the concerns regarding cardiovascular disease and COPD, and cardiovascular events and the use of long-acting bronchodilators. This chapter also provides a critical review on the existing observational studies and concludes by describing the background and rational for the present research.

Chapter three presents the study objectives. This is followed by a detailed description of the study methodologies in chapter four. The study results are presented in chapter five. In chapter six, the study conclusion, strengths and limitations and suggestions for future research are provided. Finally, chapter seven lists all references.

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Lastly, I want to thank my parents, who always love me, believe in me and encourage me through the journey.

DEDICATION

To patients who suffer from chronic obstructive pulmonary disease and cardiovascular disease.

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LIST OF ACRONYMS

ACE - Adverse Cardiovascular Event

ACE Inhibitor - Angiotensin-Converting Enzyme Inhibitor

Ach -Acetylcholine

AECOPD - Acute Exacerbation of Chronic Obstructive Pulmonary Disease

ARB - Angiotensin II Receptor Blockers

ATS - American Thoracic Society

CAD - Coronary Artery Disease

cAMP - Cyclic Adenosine Monophosphate

CI - Confidence Interval

CIIS - Cardiac Infarction Injury Score

COPD - Chronic Obstructive Pulmonary Disease

CPRD - Clinical Practice Research Datalink

CRP - C-Reactive Protein

ECG - Electrocardiogram

ED - Emergency Department

EU - European Union

ERS - Europe Respiratory Society

FDA - Food and Drug Administration

FEV - Forced Expiratory Volume

FVC - Forced Vital Capacity

GOLD - The Global Initiative for Chronic Obstructive Lung Disease Scientific Committee

HES - Hospital Episode Statistics

HR - Hazard Ratio

HRQL - Health-Related Qualify of Life

ICS - Inhaled Corticosteroids

ISAC - Independent Scientific Advisory Committee

LABA - Long-Acting β_2-Agonist

LAMA - Long-Acting Muscarinic Antagonist

MI - Myocardial Infarction

MHRA - Medicines and Healthcare products Regulatory Agency

NHS - National Health Service

NSAID - Nonsteroidal Anti-Inflammatory Drug

OR - Odds Ratio

PCI - Percutaneous Coronary Intervention

PT- Person Time

PVD - Peripheral Vascular Disease

RCT - Randomized Control Trial

RR - Rate Ratio

SABA - Short-Acting β_2-Agonist

SAMA - Short-Acting Muscarinic Antagonist

SD - Standard Deviation

SGRQ - St. George's Respiratory Questionnaire

SUMMIT - The Study to Understand Mortality and Morbidity in COPD

TDI - Transitional Dyspnea Index

TIA - Transient Ischemic Attack

TIOSPIR - Tiotropium Safety and Performance in Respimat® Trial

TORCH - TOwards a Revolution in COPD Health

TRISTAN - Trial of Inhaled Steroids and Long-acting β_2-Agonists

UK - United Kingdom

UPLIFT - The Understanding Potential Long-Term Impacts on Function with Tiotropium

US - United States

WHO - World Health Organization

CHAPTER 1 INTRODUCTION

1.1 Prevalence and economic burden of COPD

Chronic obstructive pulmonary disease (COPD) is a life-threatening lung disease and one of the leading causes of chronic morbidity and mortality worldwide (1). According to the World Health Organization (WHO), the worldwide prevalence of COPD was approximately 64 million in 2004; more than 3 million died of COPD in 2005, which accounts for 5% of all deaths worldwide (2). In the United Kingdom (UK) in 2006, approximately 835,000 people had physician diagnosed COPD, and a further estimated 2.2 million people with COPD remained undiagnosed; about 25,000 people died of COPD a year in England and Wales (3). In Canada in 2014, approximately 804,043 people accounting for 4% of the general population had physician diagnosed COPD based on a national survey conducted by Statistics Canada (4). However, more cases were suspected to be underdiagnosed and misdiagnosed (5, 6). About one in three elderly patients died of COPD within 12 months of a hospital admission for COPD according to an Ontario database study (7).

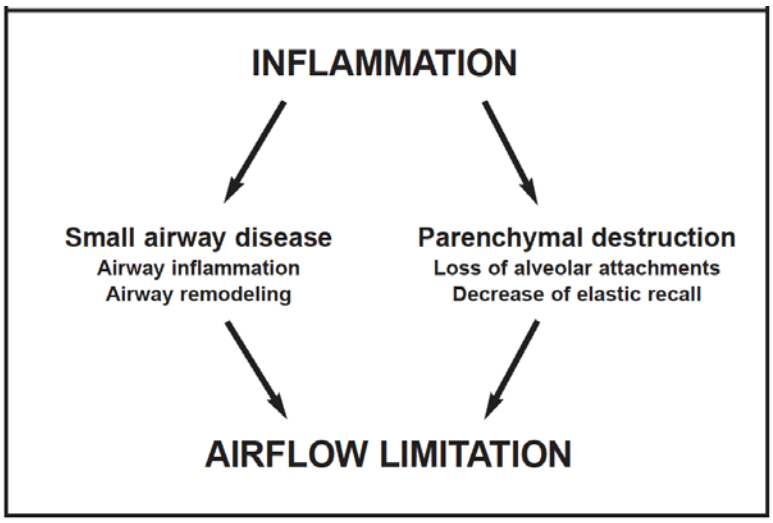
As a leading cause of morbidity and mortality, COPD contributes to a substantial economic burden on healthcare systems. In the European Union (EU), the cost of COPD (including both direct and indirect costs) was estimated as 48.4 billion Euros annually, which accounted for 50% of all cost of respiratory diseases (8). In Canada, the cost of COPD exacerbation was estimated as 1.5 billion annually (9).

1.2 Definitions, diagnosis, and natural history of COPD

It is widely accepted that COPD is characterized by "airflow limitation that is not fully reversible" (10, 11). However, there are different definitions for COPD.

The American Thoracic Society (ATS) and the Europe Respiratory Society (ERS) state that COPD is comprised of chronic bronchitis and/or emphysema, which lead(s) to airflow limitation (12, 13). The Global Initiative for Chronic Obstructive Lung Disease (GOLD) Scientific Committee avoids using terms "chronic bronchitis" and "emphysema", but explains COPD from the pathophysiology point of review. The GOLD states that an abnormal inflammation in the lung, caused by noxious particles or gases, leads to the destruction of alveoli and narrowing of the respiratory bronchioles; this, in turn, causes airflow limitation (11). Figure 1.1 illustrates the mechanisms underlying airflow limitation in COPD based on the GOLD definition.

Figure 1.1 Mechanisms underlying airflow limitation in COPD



Source. GOLD. Global Strategy for Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease. Updated 2010.

The onset of COPD occurs in patients who are 40 years or older (14). COPD is significantly underdiagnosed due to the fact that most people with airflow obstruction are not aware they have the condition (15). Patients with clinical symptoms and with a history of exposure to lung irritants, such as cigarette smoking should be considered at risk and tested for COPD. The common clinical symptoms of COPD include dyspnea, cough, sputum production, wheezing and chest tightness (11, 16), however, these clinical symptoms may not present until the later stages of the disease. These symptoms are also not specific to COPD. Therefore, Canadian guidelines (17) suggest that smokers or ex-smokers, aged 40 years or older should undertake a questionnaire screening test followed by spirometry if the screening result is positive.

Spirometry is essential for the diagnosis of COPD (18). It is performed by a device called a spirometer to measure the degree of airflow limitation. The two important measurements from a spirometry test are forced vital capacity (FVC), which measures the largest volume of air a person can blow out, and forced expiratory volume (FEV1), which measures the volume of air a person can blow out in the first second. If the ratio of these two measurements (FEV1/FVC) is below 70%, it confirms a diagnosis of COPD. Comparing the FEV1 with predicted values can further stratify the severity of COPD into mild, moderate, severe and very severe (11) (Figure 1.2).

Figure 1.2 Spirometric classification of COPD severity based on post-bronchodilator FEV1

Stage 1: Mild	$FEV_1 \geq 80\%$ predicted
Stage II: Moderate	$50\% \leq FEV_1 < 80\%$ predicted
Stage III: Severe	$30\% \leq FEV_1 \leq 50\%$ predicted
Stage IV: Very Severe	$FEV_1 < 30\%$ predicted or $FEV_1 < 50\%$ predicted plus chronic respiratory failure

FEV_1: forced expiratory volume in one second; FVC: forced vital capacity; respiratory failure: arterial partial pressure of oxygen (PaO_2) less than 8.0 kPa (60 mm Hg) with or without arterial partial pressure of CO_2 ($PaCO_2$) greater than 6.7 kPa (50 mm Hg) while breathing air at sea level. *Source*. GOLD. Global Strategy for Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease. Updated 2010.

The natural history of COPD is characterized by progressive decline in lung function (19) as a result of tobacco smoking, passive smoking, outdoor air pollution, occupational exposure to grain, isocyanates, cadmium, coal and other mineral dusts, and genetic factors such as α_1-antitrypsin deficiency (18). Additionally, exposure to indoor cooking fumes is a major cause of COPD in developing countries (20). Among all risk factors, tobacco smoking has the most important impact on COPD (21). Therefore, smoking cessation is the most effective intervention in modifying the natural history of COPD and slowing down the disease progression (18).

1.3 Management of stable COPD

COPD is preventable, and treatable at all levels of disease severity. The goals in management of COPD are to (i) relieve symptoms, (ii) improve exercise tolerance, (iii) improve health status, (iv) prevent disease progression, (v) prevent and treat exacerbations, and (vi) reduce mortality (11). The management is comprised of non-

pharmacologic and pharmacologic interventions. Both types of intervention have shown alleviation of symptoms and improvement of exercise tolerance and quality of life (17).

The most effective non-pharmacologic intervention involves avoidance of exposure to the risk factors identified earlier. For example, smoking cession is the only way to reduce COPD progression in current smokers; studies have shown that cessation can slow the accelerated decline in FEV1 (22, 23), positively affect the inflammatory process (24, 25), and reduce morbidity (26, 27) and mortality (28, 29). Oxygen therapy is another non-pharmacologic intervention that has been shown to improve reported health-related quality of life (HRQL) and increase survival in patients with severe COPD and chronic hypoxemia (30, 31). Other non-pharmacologic interventions include rehabilitation, ventilatory support and surgical treatment which all have been shown to improve exercise tolerance and relieve dyspnea (11).

Pharmacologic interventions include bronchodilators, inhaled corticosteroids, combination therapies (e.g., a combination of an inhaled corticosteroids and a long-acting β_2-agonist), and phosphodiesterase inhibitors. Although these medications have been shown to relieve the symptoms, reduce the frequency and severity of exacerbations, and improve exercise and quality of life (11, 16), none have been shown to slow disease progression or suppress inflammation or increase survival in either primary or secondary outcomes from clinical trials (28, 32, 33, 34). Although post-hoc analyses for two clinical trials have found that bronchodilators "*seemed*" to reduce the rate of decline of

FEV1 in patients with moderate to severe COPD (35, 36), this result may be limited to specific groups of patients (37).

Among the different pharmacologic interventions noted earlier, bronchodilators such as short-acting and long-acting β_2-agonists as well as short-acting and long-acting anticholinergics, are the mainstay of the pharmacologic therapy for the management of stable COPD. However, the concern regarding their cardiovascular safety remains. Although the two long-term clinical trials found that neither long-acting β_2-agonist nor long-acting anticholinergics were associated with increased risk of cardiovascular events (35, 36), several observational studies and meta-analyses have reported adverse cardiovascular effects. A detailed literature review of these clinical trials and observational studies will be presented in chapter two.

1.3.1 Acute exacerbations of COPD

Based on the GOLD guidelines, acute exacerbations of COPD (AECOPD) are defined as "an acute event characterized by a worsening of the patient's respiratory symptoms that is beyond normal day-to-day variations and leads to a change in medication". The most common causes are thought to be respiratory tract infections, either viral or bacterial (11). Other factors include congestive heart failure, exposure to allergens and irritants, and pulmonary embolism (17). Currently, there has been no biomarker developed for a precise etiologic diagnosis. The diagnosis of an acute exacerbation relies exclusively on clinical presentation of a group of worsening respiratory symptoms, such as acute worsening dyspnea, cough and/or sputum production (11).

Pharmacologic treatment for AECOPD usually involves bronchodilators, corticosteroids and antibiotics. Short-acting β_2-agonists (SABA), or combined SABA and a short-acting anticholinergics are typically considered the first-line therapy to treat AECOPD. For patients with moderate or severe AECOPD, oral corticosteroids (prednisone with a daily dosage of 25 to 50 mg for between five and fourteen days) are recommended. Antibiotics are only recommended for exacerbations that are associated with a history of more purulent sputum (11, 17, 38).

CHAPTER 2 LITERATURE REVIEW

2.1 Bronchodilators for patients with COPD

Various bronchodilators are the mainstay of the pharmacologic therapy for COPD. The available bronchodilators include β_2-agonists, anticholinergics, and combination bronchodilator therapies.

2.1.1 β_2-agonists

The primary effect of a β_2-agonist in COPD is to dilate the bronchi by binding to β_2 receptors, which are significantly present in the airway smooth muscle cells. These receptors promote an increase in cyclic adenosine monophosphate (cAMP); cAMP, in turn, stimulates the relaxation of bronchial muscles (39). The β_2-agonists are usually administered via inhalation of aerosol, dry powder or nebulized solution (40).

2.1.1.1 Short-acting β_2-agonists

Two types of β_2-agonists are short-acting and long-acting agents. A short-acting β_2-agonist (SABA) usually has a rapid onset of action, typically beginning within 3 minutes with peak activity after 2.5 hours (41). Its therapeutic duration of action is from 4 to 6 hours, and it is typically recommended as an initial treatment for mild COPD, but is also used in both stable and acute management of COPD (11). Results from a 2002 Cochrane Systematic Review that included 13 randomized control trials (RCTs) showed that use of SABA resulted in a slight but significant improvement in lung function and in daily breathlessness score when compared to placebo. However, none of the selected trials

reported reliable information on adverse effects due to limited sample size and short duration of follow-up (42).

2.1.1.2 Long-acting β_2-agonists

Long-acting β_2-agonists (LABAs) are typically recommended as a maintenance therapy for patients with stable COPD. Compared with SABAs, LABAs are more effective and convenient, and can provide more sustained improvement in pulmonary function (e.g., FEV1), dyspnea and health-related qualify of life (11). Additionally, a meta-analysis showed that in general LABAs significantly reduced COPD exacerbations when compared to placebo (43).

2.1.1.2.1 Salmeterol and formoterol

The most commonly used LABAs are salmeterol and formoterol. Both have a therapeutic duration of action of 12 hours after inhalation of a single dose. Therefore, only a twice-daily treatment frequency is required, which is particularly useful for patients to relieve nighttime or early morning symptoms. When compared to salmeterol, formoterol has been shown to have a faster onset of action due to its lower lipophilicity. It can stimulate bronchodilation within 1 to 3 minutes, similar to that of SABAs, while salmeterol's onset of action is at least 20 minutes. In addition, formoterol is more potent than salmeterol due to the fact that formoterol is a full β_2-agonist, while salmeterol is a partial β_2-agonist. The potential clinical benefit of a full β_2-agonist is that it can fully activate β_2 receptors and promote better bronchodilation. However, a full agonist may produce a more rapid desensitization and may potentially cause more adverse events when compared to a

partial β_2-agonist. (41) The potential adverse events of a full agonist include tachycardia and reduction in serum potassium (44). Despite these characteristics, 50 μg salmeterol has been demonstrated to have a comparable effect to 12 μg formoterol in a short-term reversal of airway obstruction in patients with mild to severe COPD (45).

Salmeterol was the first long-acting bronchodilator licensed by authorities to treat patients with COPD. It was approved by the Medicines and Healthcare products Regulatory Agency (MHRA) in the UK in 1996 and the Food and Drug Administration (FDA) in the United States (US) in 1998. Formoterol has been available since 2001 in both the UK and US (39). Since then, there have been several clinical trials conducted to evaluate the efficacy and safety of these two β_2-agonists in COPD. Although, there were no head to head clinical trials conducted to compare the efficacy of these two β_2-agonists directly, it has been found that both formoterol and salmeterol demonstrated clinical benefits in improving lung function and reducing exacerbation rate when compared to placebo, or ipratropium, or theophylline.

Boyd G et al, 1997 (46) examined the efficacy and safety of salmeterol (50 and 100μg *b.i.d.*) versus placebo in a 16-week RCT in 674 patients with COPD. The authors found that either salmeterol 50μg or 100μg *b.i.d.* group showed a significant improvement in respiratory symptoms and lung function (e.g., FEV1) when compared with placebo. Both salmeterol groups showed a reduced use of additional daytime salbutamol. The most commonly reported adverse events were COPD exacerbations, headache and tremor.

Other than tremor being reported significantly higher in the salmeterol 100 μg *b.i.d.* group, adverse events were comparable across all three groups.

van Noord et al, 2000 (47) compared the efficacy and safety of salmeterol alone with the combination of salmeterol plus ipratropium and with placebo in a 12-week RCT in 144 patients with severe stable COPD. Patients in the salmeterol alone group showed a significant increase in FEV1, however, the combination of salmeterol plus ipratropium demonstrated a greater improvement in FEV1. Additionally, both salmeterol alone and the combination of salmeterol plus ipratropium groups were associated with a significant improvement in daytime symptoms and morning peak expiratory flow compared with placebo. Although, both active treatments were well tolerated, the most common adverse events reported were headache, cough, and COPD exacerbations.

Table 2.1 provides an overview of the clinical trials conducted pertaining to the efficacy of salmeterol. Any potential adverse cardiovascular events are also summarized.

Table 2.1 Overview of clinical trials of efficacy and cardiovascular safety of salmeterol

Studies	Comparators	Sample size (N) and follow-up duration	Outcomes	Adverse cardiovascular events (ACEs)
Salmeterol				
Boyd 1997 (46)	Salmeterol 50µg; Salmeterol 100µg; Placebo	N=674 16 weeks	Increase in FEV1 AEs were similar in all groups.	Although electrocardiography (ECG) was a safety endpoint at baseline and at the end of treatment, no ACE was reported.
Jones 1997 (48)	Salmeterol 50µg; Salmeterol 100µg; Placebo	N=283 16 weeks	Increase in FEV1 Improve in SGRQ	No ACE was reported.
Mahler 1999 (49)	Salmeterol 42µg; Ipratropium 36µg; Placebo	N=411 12 weeks	Increase in FEV1 Reduce dyspnea and use of rescue med Longer time to exacerbation AE were similar in all groups.	No ACE was reported.
van Noord 2000 (47)	Salmeterol 50µg; Salmeterol/ipratropium 5µg /40µg; Placebo	N=144 12 weeks	Increase in FEV1 Improve daytime symptoms and morning peak expiratory flow Reduce use of rescue med	No ACE was reported.
Cazzola 2000 (50)	Salmeterol 50µg; Salmeterol/fluticasone 50µg /250µg; Salmeterol/fluticasone 50µg /500µg; Salmeterol/theophylline 50µg plus	N=80 12 weeks	Increase in FEV1	Patients with unstable angina or arrhythmias were excluded; no ACE was reported at the end of the study.
Rennard 2001 (51)	Salmeterol 42µg; Ipratropium 36µg;	N=405 12 weeks	Increase in FEV1	Patients with cardiovascular disease or abnormal ECG were excluded; no

Studies	Comparators	Sample size (N) and follow-up duration	Outcomes	Adverse cardiovascular events (ACEs)
	Placebo			ACE was reported at the end of the study.
ZuWallack 2001 (52)	Salmeterol 42µg; Salmeterol/ theophylline 42µg; Theophylline	N=943 12 weeks	Increase in FEV1 Improve treatment satisfaction Fewer drug-related adverse events	ACEs were reported from 1 to 4% (relatively rarely) and were similar among all treatment groups, not statistically significant. Most frequently reported serious ACEs included rhythm disturbances, congestive failure, or myocardial infarction (MI), but not statistically significant.
Chapman 2002 (53)	Additional salmeterol 50µg on concurrent anticholinergic therapy; Additional placebo on concurrent anticholinergic therapy	N=408 24 weeks	Increase in FEV1 Improve morning peak flow Few exacerbation of COPD	No ACE was reported.
Mahler 2002 (54)	Salmeterol/fluticasone 50/500 mcg; Fluticasone 500 mcg; Salmeterol 50 mcg; Placebo	N=691 24 weeks	Increase in FEV1 Reduce the severity of dyspnea	Although ECG and 24-hour Holter monitor were safety endpoints, the incidence of abnormal ECG was comparable among treatment groups; no unexpected ACE was reported.
Calverley 2003 (55) (TRISTAN)	Salmeterol 50µg; Fluticasone 500µg; Salmeterol/ fluticasone 50µg/ 500µg	N=1465 52 weeks	Increase in FEV1 Improve health status Reduce use of rescue med Reduce freq. of exacerbation	No ACE was reported.
Calverley 2007 (TORCH) (56)	Salmeterol 50µg; Fluticasone 500µg; Salmeterol/ fluticasone 50µg/	N=6112 3 years	Increase in FEV1 Improve health status Reduce the rate of	Deaths due to cardiovascular causes were reported comparable among treatment groups.

Studies	Comparators	Sample size (N) and follow-up duration	Outcomes	Adverse cardiovascular events (ACEs)
	500μg; Placebo		exacerbations	

Dahl R et al, 2001 (57) found in a 12-week randomized, double-blind, parallel-group study that patients receiving formoterol had a significant improvement in respiratory symptoms compared to patients on ipratropium. Overall respiratory symptoms were measured by patient-completed diary which consisted of 4 items related to ability to perform usual daily activities, breathlessness over the past 24 hours, waking at night due to respiratory symptoms, breathlessness when waking up, and coughing and sputum production. Similar adverse cardiovascular events (ACEs) were reported among treatment groups; heart rate and rhythm disorders were uncommon cross treatment groups; one patient from formoterol 12 μg group had a significant electrocardiogram (ECG) alteration after 12 weeks compared to that at baseline, and suffered from atrial fibrillation.

Aalbers R et al. 2002 (58) also reported that formoterol (at a dose of 18 μg *b.i.d.*) was associated with greater improvement in respiratory symptoms compared with placebo. The improvement was less in formoterol at doses 4.5 μg or 9 μg *b.i.d.* Symptoms of COPD were accessed by using Transitional Dyspnea Index (TDI), which evaluates the impact on daily activities of breathlessness. The most common adverse events were deterioration of COPD, respiratory infection, hypertension, and tachycardia, but none of these events showed a relation to formoterol treatment.

Table 2.2 provides an overview of the clinical trials conducted pertaining to the efficacy of formoterol. Any potential adverse cardiovascular events are also summarized.

Table 2.2 Overview of clinical trials of efficacy and cardiovascular safety of formoterol

Studies	Comparators	Sample size (N) and follow-up duration	Outcomes	Adverse cardiovascular events (ACEs)
Formoterol				
Dahl 2001 (57)	Formoterol 12μg; Formoterol 24μg; Ipratropium 40μg; Placebo	N=780 12 weeks	Increase in FEV1 Improve symptoms and quality of life AEs were similar in all groups	ACEs were reported comparable among treatment groups; heart rate and rhythm disorders were uncommon; only 1 patient from formoterol 12μg group had significant ECG alteration after 12 weeks compared to that at baseline.
D'Urzo 2001 (59)	Formoterol/ ipratropium 12μg / 40μg; Salbutamol/ ipratropium 200μg / 40μg	N=272 3 weeks	Improve morning peak expiratory flow Increase in FEV1 Improve symptoms AEs were similar in both groups	ECG, blood pressure and heart rate were safety endpoints. Only 1 patient had congestive heart failure at the end of the study in salbutamol/ipratropium combination group.
Rossi 2002 (60)	Formoterol 12μg; Formoterol 24μg; Theophylline	N=854 52 weeks	Increase in FEV1 Improve peak expiratory flow Reduce use of rescue med	Heart/rhythm disorders were reported rarely and comparable among treatment groups. No deaths related to cardiac adverse events were reported.
Aalber 2002 (58)	Formoterol 4.5μg; Formoterol 9μg; Formoterol 18μg; Placebo	N=692 12 weeks	Increase in FEV1 Improve symptoms and reduce use of rescue med No unexpected adverse events	Patients with significant or unstable heart disease were excluded; ECG, heart rate and blood pressure were safety endpoints; no ACE was reported.
Calverley 2003 (61)	Formoterol/prednisolone 9μg / 30mg; Inhaled budesonide/formoterol	N=1022 12 months	Increase in FEV1 Prolonged time to first exacerbation	Patients with cardiovascular disorder were excluded. Only few deaths were related to ACE.

Studies	Comparators	Sample size (N) and follow-up duration	Outcomes	Adverse cardiovascular events (ACEs)
	320/9μg; Budesonide 400μg; Formoterol 9μg; Placebo			
Szafranski 2003 (62)	Budesonide/formoterol (Symbicort) 160/4.5μg; Budesonide 200μg; Formoterol 4.5μg; Placebo	N=812 16 weeks	Reduce freq. of severe exacerbation Increase in FEV1 Improve morning and evening peak expiratory flow Decrease symptom and use of rescue med Improve health related quality of life	ACEs were reported comparable among treatment groups.

2.1.2 Anticholinergics

The anticholinergic bronchodilators are also referred to as muscarinic antagonists. The mechanism of action of anticholinergics occurs through antagonism of acetylcholine (Ach) at M3-muscarinic receptors located in bronchial smooth muscle cells, submucosal mucus glands, and vascular endothelium in the lung (41). Besides M3-muscarinic receptors, there are 4 other distinct G-protein coupled receptors (M1, M2, M4 and M5) mediated by Ach. Although the role of M4 and M5 is still unknown, M1 receptors are located in parasympathetic ganglia and play a role in simulating neurotransmission; M2 receptors act as autoreceptors and are found in the post ganglionic para-sympathetic nerve to promote Ach release. In addition, the stimulation of M2 receptors is associated with negative chronotropic and inotropic effects, and inhibition of M2 receptors through antagonism of muscarinic receptors may cause tachycardia. Therefore an optimal muscarinic antagonist should selectively block only M3 and/or M1 receptors (63).

As for the β_2-agonists, muscarinic antagonists also have either short-acting or long-acting agents. The most commonly used short-acting muscarinic antagonists (SAMA) are ipratropium and oxitropium (this medication was discontinued in 2004) (41). Ipratropium has an effect on all muscarinic receptors rather than selectively blocking only M1 and M3 receptors. The onset of action of ipratropium occurs within minutes. Its peak activity duration is between 1 and 4 hours, which favors treatment frequency of two inhalations four times daily. (41) As shown in the previous section, when compared to LABAs, ipratropium appears to be inferior in terms of reducing exacerbations and improving lung function and health-related quality of life (49, 51, 57). However, when it is given with

LABA as a combination therapy, the combination of a LABA and ipratropium has shown statistically significant clinical benefits in increase in FEV1 and symptom improvement when compared to LABA alone, or ipratropium alone (47, 59).

2.1.2.1 Tiotropium

The most commonly used long-acting muscarinic antagonist (LAMA) is tiotropium, which is the first approved LAMA and available since 2002 in the UK and 2004 in the US (39). The binding affinity of tiotropium to M1, M2, and M3 receptors is similar to that of ipratropium. However, tiotropium dissociates more slowly from M1 and M3 receptors, which leads to its long-lasting effect (64). It can achieve bronchodilation within 30 minutes, and sustain this over more than 24-hours; therefore, only a once-daily dose is required. The most common adverse event is dry mouth, which is a result of inhibited salivary section through blocking M1 and M3 receptors. Tiotropium can be given in a dry powder Handihaler® device at 18μg once daily (the device was approved in 2002 in the UK). It is also available in another device called Respimat® (propellant-free liquid inhaler) at a dose of 2 inhalations (2.5 μg each) of the spray daily (the device was available in 2007, but only received its approval from MHRA in the UK in July 2012).

Several clinical trials have evaluated the efficacy of tiotropium in Handihaler® and Respimat®. Although the follow-up duration was only up to 4 weeks, earlier clinical trials (65, 66) demonstrated similar clinical benefits with tiotropium Handihaler® 18μg and Respimat® 2.5 μg in improving lung function, symptoms and quality of life.

The most recent clinical trial (TIOSPIR) (67) was conducted to compare both efficacy and safety of tiotropium in these two different devices. 17,135 participants were randomized into to three treatment groups to receive tiotropium Respimat® 5µg once daily, 2.5µg once daily and tiotropium Handihaler® 18µg once daily. After a follow-up duration of 2.3 years, it was found that tiotropium Respimat® and tiotropium Handihaler® are comparable in terms of safety profile and prevention of exacerbations. The hazard ratios of all-cause mortality were reported to be 0.96 (95% CI: 0.84 to 1.09) when comparing Respimat® 5µg to Handihaler® 18µg; and 1.00 (95% CI: 0.87 to 1.14) when comparing Respimat® 2.5 µg to Handihaler® 18µg. The reported hazard ratio of first exacerbation was 0.98 (95% CI: 0.93 to 1.03) in comparison between Respimat® and Handihaler®. The study also concluded that major adverse cardiovascular events were similar in all three treatment groups. However, the study excluded patients with previous cardiovascular events, such as myocardial infarction within the previous 6 months, hospitalization for class III or IV heart failure, or unstable or life-threatening arrhythmia requiring new treatment within 12 months. Additionally, with absence of placebo group, the results cannot imply any association between the use of tiotropium and all-cause mortality nor cardiovascular safety.

In the 4-year UPLIFT trial (68), Tashkin and colleagues examined the long-term effects of tiotropium therapy as compared to placebo in 5,993 patients with COPD. The investigators found that although the improvement in FEV1 with tiotropium was maintained throughout the trial when compared to placebo, tiotropium did not slow the rate of decline in the FEV1. Investigators also found that tiotropium demonstrated

significant improvement in quality of life and reduction of exacerbations during 4-year period. The results are similar to those found in previous trials, but demonstrate a long-term clinical benefit of tiotropium. Although adverse cardiovascular events were not within the primary or secondary objectives, the study found that tiotropium was not associated with an increase in major cardiovascular events. It reported that 152 patients developed myocardial infarction, 67 were in the tiotropium group and 85 were in the placebo group (relative risk, 0.73; 95% CI, 0.53 to 1.00). 82 patients in the tiotropium group and 80 in placebo group developed stroke (relative risk, 0.95; 95% CI, 0.70 to 1.29). In addition, tiotropium was reported to be associated with a lower incidence rate of serious adverse events, including congestive heart failure, compared to placebo.

Table 2.3 Overview of clinical trials of efficacy and cardiovascular safety of tiotropium

Studies	Comparators	Sample size (N) and follow-up Duration	Outcomes	Adverse cardiovascular events (ACEs)
Tiotropium				
Casaburi 2002 (69)	Tiotropium 18μg; Placebo	N=921 1 year	Increase in FEV1 Reduce dyspnea, freq. of exacerbation and hospitalization Improve health status	Patients with history of myocardial infarction (≤ 1yr), heart failure (≤ 3yrs) or cardiac arrhythmia were excluded; No ACE was reported.
Donohue 2002 (70)	Tiotropium 18μg; Salmeterol 50μg; Placebo	N=623 6 months	Tiotropium is superior to salmeterol in improving lung function (increase in FEV1), dyspnea and quality of life	A cardiac arrest was reported in placebo group.
Vincken 2002 (71)	Tiotropium 18μg; Ipratropium 40μg	N=535 1 year	Increase in FEV1 Improve dyspnea, exacerbation, and quality of life	Although ECG was a safety endpoint, no abnormal ECG was observed that could be attributed to tiotropium.
Brusasco 2003 (72)	Tiotropium 18μg; Salmeterol 50μg; Placebo	N=1207 two 6-month RCTs	Tiotropium prolonged time to first exacerbation when compared to placebo; Reduced freq. of exacerbation; Improved quality of life, dyspnea, and lung function (increase in FEV1)	Two deaths due to cardiac arrest were reported in placebo group.
Briggs (2005) (73)	Tiotropium 18μg; Salmeterol 50μg	N=653 12 weeks	Increase in FEV1	No ACE was reported.
Tashkin (UPLIFT) (2008) (68)	Tiotropium 18μg; Placebo	N=5993 4 years	Increase in FEV1 Improve quality of life, exacerbation; not statistically significant on disease progress (reduce the rate of decline in	Tiotropium was associated with a reduction in cardiac adverse events.

Studies	Comparators	Sample size (N) and follow-up Duration	Outcomes	Adverse cardiovascular events (ACEs)
			FEV1)	
Vogelmeier (POET) (2011) (74)	Tiotropium 18µg; Salmeterol 50µg	N=7376 1 year	More effective in preventing exacerbations.	Comparable ACEs were reported in both treatment groups.

In the 1-year POET trial (74), tiotropium was compared with salmeterol in preventing exacerbations of COPD. Vogelmeier et al found that tiotropium significantly prolonged the time to the first exacerbation and reduced the annual number of severe exacerbations when compared to salmeterol. However, no difference was found between the two groups in regards to the incidence of serious adverse events and for adverse events leading to the discontinuation of treatment.

Several clinical trials (70, 72, 74) have demonstrated that tiotropium can be more effective than a long-acting β_2-agonist, such as salmeterol, in reducing the number of exacerbations and increasing the time to first exacerbation. However, various treatment guidelines (11, 17, 38) make no distinction as to which long-acting bronchodilator should be recommended as an initial treatment. The GOLD guidelines suggest that the choice of treatment should rely on patient's response in symptom relief and adverse events (11).

2.1.3 Combination therapy

There are two fixed-dose combination inhalers available to treat patients with COPD. Both are a combination of a LABA agent and a corticosteroid (ICS), salmeterol/fluticasone and formoterol/budesonide. Salmeterol/fluticasone, brand name Seretide®, has been available in the UK since 2000; formoterol/budesonide, brand name Symbicort®, has been available in the UK since 2001. LABA/ICS combinations have been shown to improve pulmonary function (FEV1) and health-related quality of life, relieve respiratory symptoms, reduce the rate of exacerbation and prolong time to first exacerbation in patients with COPD.

Calverley P et al. 2003 (55) examined the combination of salmeterol and fluticasone versus either salmeterol alone, fluticasone alone, or placebo in a one-year randomized control trial (TRISTAN) in 1,465 patients with COPD. Authors found that all active treatments, including salmeterol, fluticasone and salmeterol plus fluticasone, statistically improved lung function (FEV1) when compared to placebo. Additionally, the combination of salmeterol and fluticasone reduced the rate of exacerbations by 25% ($p<0.0001$), such a reduction was significantly larger than that of either monotherapy groups (20%, $p=0.0027$ in salmeterol group, and 19%, $p=0.0033$ in fluticasone group). This combination therapy also improved health status and relieved daily symptoms significantly. However, no difference in the frequency of adverse events, such as serum cortisol concentration, skin bruising, or ECGs, was found among all four treatment groups.

Another study by Calverley PM et al. 2003 (61) suggested a similar effect of formoterol combined with the inhaled corticosteroid budesonide in relieving symptoms and improving health-related quality of life (HRQL) in a 12-month randomized, double-blind, parallel-group study. The HRQL was assessed by the St George's Respiratory Questionnaire (SGRQ), which is a patient-reported measure that consists of 40 items with domains of symptoms, activities that cause breathlessness, and impacts on daily life. The study also reported a prolonged time to first exacerbation for patients receiving combination therapy of formoterol/budesonide compared with patients on placebo.

The effects of salmeterol/fluticasone, salmeterol alone, fluticasone alone and placebo on survival have been examined in a long-term (3-year) randomized, double blind, placebo-controlled clinical trial study design (56). In the study (TORCH) by Calverley P et al, salmeterol plus fluticasone demonstrated the lowest mortality rate among all four treatment groups, although a non-significant hazard ratio for death in the combination therapy was reported when compared to placebo (0.83, 95% CI 0.68 to 1.00). The mortality rate was not different among salmeterol, fluticasone, or placebo groups. The TORCH trial also confirmed similar findings reported in previous trials with short follow-up duration. They found that salmeterol reduced the rate of exacerbations, hospitalizations, and improved health status, and spirometric measurements over the 3-year study duration. The combination therapy of salmeterol and fluticasone demonstrated a larger clinical benefit than that observed in salmeterol or fluticasone alone groups. In addition, no excess of cardiac disorders were found in either combination therapy group or monotherapy groups.

Table 2.4 summarizes clinical trials pertaining to the efficacy and cardiovascular safety profile of combination therapies.

Table 2.4 Overview of clinical trials of efficacy and cardiovascular safety of combination therapies

Studies	Comparators	Sample size (N) and follow-up duration	Outcomes	Adverse cardiovascular events (ACEs)
Mahler 2002 (54)	Salmeterol/fluticasone 50 and 500 mcg; Fluticasone 500 mcg; Salmeterol 50 mcg; Placebo	N=691 24 weeks	Increase in FEV1 Reduce the severity of dyspnea	Although ECG and 24-hour Holter monitor were safety endpoints, the incidence of abnormal EDC was comparable among treatment groups; no unexpected ACE was reported.
Calverley 2003 (55) (TRISTAN)	Salmeterol 50μg; Fluticasone 500μg; Salmeterol/fluticasone 50/ 500μg	N=1465 52 weeks	Increase in FEV1 Improve health status Reduce use of rescue med Reduce freq. of exacerbation	No ACE was reported.
Calverley 2007 (TORCH) (56)	Salmeterol 50μg; Fluticasone 500μg; Salmeterol/fluticasone 50/ 500μg ; Placebo	N=6112 3 years	Increase in FEV1 Improve health status Reduce the rate of exacerbations	Deaths due to cardiovascular causes were reported comparable among treatment groups.
Calverley 2003 (61)	Formoterol 9μg plus oral prednisolone 30mg; Inhaled budesonide/formoterol 320/9μg; Budesonide 400μg; Formoterol 9μg; Placebo	N=1022 12 months	Increase in FEV1 Prolonged time to first exacerbation	Patients with cardiovascular disorder were excluded. Only few deaths were related to ACE.
Szafranski 2003 (62)	Budesonide/formoterol (Symbicort) 160/4.5μg; Budesonide 200μg; Formoterol 4.5μg; Placebo	N=812 16 weeks	Reduce freq. of severe exacerbation Increase in FEV1 Improve morning and evening peak expiratory	ACE was reported comparable among treatment groups.

Studies	Comparators	Sample size (N) and follow-up duration	Outcomes	Adverse cardiovascular events (ACEs)
			flow Decrease symptom and use of rescue med Improve health related quality of life	
Wedzicha 2008 (75)	Salmeterol/fluticasone, 50/500 μg; Tiotropium 18 μg	N=1,323 2 years	No difference in the rate of exacerbations between salmeterol/fluticasone and tiotropium; The rate of mortality was significant lower in the salmeterol/fluticasone group. When compared to tiotropium group.	ECG was performed at week 0, 56, and 104; less than 2% of patients had clinically significant ECG abnormalities in either of the treatment groups.

Free combinations of LABA and LAMA are recommended to patients whose COPD is not sufficiently controlled with monotherapy (11, 17, 38). The combination could potentially maximize the bronchodilator responses without increasing the dose of either drug (76, 77). Several studies (78, 79, 80) have investigated this combination and showed that it can improve lung function and reduce COPD exacerbations. Such combinations are now available in a single inhaler.

2.1.4 A brief summary of reviewed clinical trials of long-acting bronchodilators

Although clinical benefits are well demonstrated, long-acting bronchodilators have not been shown to slow the progression of COPD. Data reported in the prospective UPLIFT trial (68) failed to show a significant reduction in the rate of decline in mean FEV_1. Additionally, no data were shown that long-acting bronchodilators can reduce mortality. In the TORCH trial (56), the mortality rate was not reported significantly different from the placebo group for salmeterol alone or fluticasone alone.

Moreover, clinical trials have not assessed the association between adverse cardiovascular events and the use of long-acting bronchodilators as the primary or secondary study objectives; and have failed to demonstrate as to whether there is an increased risk of cardiovascular events associated with the use of long-acting bronchodilators. Results may be limited due to a short follow-up duration, and most importantly, the exclusion of patients with history of prior cardiovascular events, especially for patients with abnormal ECG, arrhythmia and myocardial infarction. Two randomized control trials (35, 36) with longer durations did not found a statistically

significant association between the use of long-acting bronchodilators and risk of cardiovascular events, and it may have suffered from limitations in methodology such as inclusion of prevalent users of the study drug before randomization, exclusion of subjects with prior cardiovascular disease, invalid factorial statistical analysis (81), and post-hoc analysis (82).

2.2 Myocardial infarction – a brief overview

Myocardial infarction (MI), commonly known as a heart attack, is a leading health problem and thus also an outcome measure in many clinical trials and observational studies. MI is caused by thrombus and acute plaque rupture in the coronary artery, which results in a sudden disruption of blood flow, leading to lack of oxygen supply, and consequently damage to the heart muscle (83). Clinical symptoms of an acute MI include sudden chest pain, shortness of breath, sweating, nausea, vomiting, abnormal heartbeats and anxiety. However, these symptoms are not specific for MI and can be misdiagnosed. The differential diagnosis includes gastrointestinal, neurological, pulmonary, or musculoskeletal disorders. In addition, some MIs may occur with atypical symptoms, or without symptoms (84). Therefore, an electrocardiogram (ECG), laboratory tests, or cardiac imaging are the main methods of diagnosing an MI.

Risk factors of an MI include a family history of cardiovascular disease, older age, male sex, smoking, diabetes, hypertension, obesity, and hypercholesterolemia (85). The management for MI initiated in the hospital, so patients can receive continuous cardiac monitoring. Percutaneous coronary intervention (PCI) can be used to restore flow in the

occluded artery. Fibrinolytic drugs should be administered if PCI cannot be given within 90 minutes of diagnosis. Other commonly used medications for patients with MI include antiplatelet agents, beta blockers, angiotensin-converting enzyme inhibitors, cholesterol-lowering agents (86).

MI is a leading cause of mortality and morbidity worldwide. It is reported approximately 72,750 people died from cardiovascular disease in Canada in 2004. The incidence of MI was 241.6 per 100,000 in men and 135.7 per 100,000 in women in 2005/2006, costing the Canadian healthcare system approximately $8.1 billion in 2000 (87). In the UK, approximately 80,000 people die from coronary heart disease each year. In England, the incidence of MI was 154 per 100,000 in men and 34 per 100,000 in women in 2010, costing the UK healthcare system approximately £8.7 billion in 2009 (88).

2.3 Cardiovascular disease and COPD

Cardiovascular disease is common and is the leading cause of mortality among patients with COPD. Both cardiovascular disease and COPD share important risk factors such as tobacco smoking and older age. Early prospective cohort studies showed that impaired pulmonary function, characterized by reduced expiratory flow volumes, was an important risk factor for ischemic heart disease, myocardial infarction, stroke, and sudden cardiac death (89, 90, 91). The mechanism of this synergy, however, remains largely unknown.

Based on recent studies, it is increasingly recognized that COPD is characterized by systemic inflammation (11). Noxious inhaled gases or particles from tobacco smoking

provoke an inflammatory response of the lung. One of the inflammatory responses is elevated C-reactive protein (CRP), which is linked to an increased risk of cardiovascular events (92).

Sin DD et al. (2003) (92) conducted an analysis of the 3rd National Health and Nutrition Examination Survey (NHANES) (N=6,629) to explore whether CRP and other systemic inflammatory markers were present in participants with COPD and were associated with cardiac injury. The Cardiac Infarction Injury Score (CIIS), an electrocardiogram classification system, was used to estimate the risk of underlying ischemic heart disease. They found that participants with severe pulmonary obstruction were more likely to have an increased circulating CRP level. The calculated CIIS was 2.68 and 5.88 higher in patients with highly elevated CRP and impaired lung function compared to patients with low CRP and patients without impaired lung function, respectively. They concluded that low-grade systemic inflammation found in patients with moderate to severe pulmonary obstruction was associated with the increased risk of cardiac injury.

2.4 Cardiovascular events and long-acting bronchodilators

As summarized in pervious sections, clinical trials have not demonstrated that an increased risk of cardiovascular events is associated with the use of long-acting bronchodilators. However, observational studies and meta-analyses found that β_2-agonists and inhaled tiotropium led to significantly increased adverse cardiovascular events in patients with COPD. To date there have been several observational studies which have examined the potential association.

2.4.1 Critical appraisal of observational studies

Below is a critical appraisal of those observational studies which examined the association between the use of long-acting bronchodilators and acute MI or other cardiovascular events in patient with COPD.

Verhamme, et al (2012)

Verhamme et al (93) identified a cohort of 6,788 subjects 40 years of age or older that included both "prevalent" and "incident" subjects with COPD from January 2000 to May 2007 using the Dutch healthcare database. Prevalent COPD was defined as a COPD diagnosis prior to cohort entry period while incident COPD was defined as the first COPD diagnosis during study follow-up. As a result, a total of 784 cardiovascular or cerebrovascular events were identified, and were matched on index date, gender and year of birth with 25,899 control moments selected from the cohort. The association between the use of LABA or tiotropium Handihaler and cardiovascular or cerebrovascular events was estimated using models for matched data for the nested case-control approach. Overall, they concluded that the current use of tiotropium Handihaler was not associated with an increased risk of a cardiovascular or cerebrovascular event when compared to the current use of LABA. The adjusted odds ratio was 0.89 (95% CI: 0.55 to 1.44). This study has several methodological limitations which may have impacted its validity.

The first methodological concern is that the authors failed to conduct this research in a new-user cohort. The cohort entry date was defined as the first date of diagnosis of COPD for incident COPD subjects and the date of study entry (January 2000) for prevalent COPD subjects, with only 23% of the 6,788 subjects having incident COPD. As

such, many subjects initiated LABAs or tiotropium prior to diagnosis and/or cohort entry, so that the study cohort is a differential mix of new and prevalent users of LABAs or tiotropium, with more prevalent users of LABAs than tiotropium since LABAs have been on the market for longer. Therefore, the study is vulnerable to bias from depletion of susceptibles due to the fact that the risk of an outcome associated with a therapeutic agent may vary over time (94). Patients who remained on an agent are likely to be those who can tolerate the agent better than those who are susceptible. A previous study conducted by *Au et al* (95) showed there was a sevenfold increase in the risk of MI in patients with an initial prescription of an inhaled β_2-agonist within 3 months prior to their event date. Therefore, in this study, patients who were prevalent users of LABAs or tiotropium were more likely to be selected as controls, thus underestimating the risk associated with the use of LABAs or tiotropium. Furthermore, inclusion of prevalent users may also lead to over-adjustment for COPD risk factors that may be affected by initial exposure to the treatment (96). A confounding variable is usually measured just prior to cohort entry, but with prevalent cohort may be measured in the pathway between respiratory medication exposure and the adverse cardiovascular or cerebrovascular events.

The second methodological issue pertains to insufficient matching strategy. The study was only matched on index date, gender and age at birth. Since the study cohort is a differential mix of incident and prevalent COPD subjects, the authors should have further matched on COPD duration, as adjusting COPD duration in the multivariate logistic regression may not be sufficient.

The third issue pertains to the unclear exposure definition. The study aimed to evaluate whether current use of tiotropium Handihaler may increase the risk of cardiovascular or cerebrovascular events when compared to the current use of LABAs. However, besides exposure categories for current use of tiotropium and LABA, the study defined 6 other exposure categories, among them "current use combinations of drug classes" (further defined in the footnote from Table 3 in the study "combinations of fixed or individual respiratory preparations other than SABA") may have included fixed combination therapy of ICS/LABA, or free combination of a LABA and tiotropium. This may result in misclassification of exposure status. The authors could have categorized the fixed combination therapy of ICS/LABA into the current use of LABA group and further adjusted for ICS use in the analysis, and created another exposure category for combination use of a LABA and tiotropium.

Additionally, the subjects may have contributed multiple times to the case group. The index date was defined as the first cardiovascular or cerebrovascular event since cohort entry. Usually a nested case-control study will end at index event, and control will be selected from subjects who were still disease-free at index date. However, this study may have continued identifying cases beyond the first occurrence, as can be observed when counting the cases. In Table 3 and Results section 3.2, it is reported that 784 cases of cardiovascular or cerebrovascular event were identified, which was consisted of 254 cases of stroke and transient ischemic attacks (TIA), 116 cases of MI, 413 cases of heart failure and 6 cases of ventricular arrhythmia. If we sum the cases of each endpoint, it gives us a total of 789 cases. In Table 4, where the authors performed a sensitivity

analysis for separate endpoints, they further reported 357 cases of stroke and TIA, 155 cases of MI and 446 cases of heart failure, which summed up as 958 cases. Such of recurring cardiovascular or cerebrovascular events may affect subsequent exposure and thus introduce bias in the analysis.

Jara M, et al (2012)

Jara et al (97) published a study entitled, "A new user cohort study comparing the safety of long-acting inhaled bronchodilators in COPD". They assessed the incidence rates of total stroke, MI, angina and other adverse events among new users of tiotropium (via Handihaler) versus new users of long-acting β_2-agonist monotherapy in a retrospective cohort study design. The source population was subjects from The Health Improvement Network database in the UK. Incidence rates of stroke, MI, angina and other adverse events were calculated as number of subjects experiencing an event divided by the person-years at risk. Hazard ratios of the adverse events were calculated by using Cox proportional hazard analysis. As a result, the authors found that tiotropium was not associated with a statistically significant higher rate of stroke (hazard ratio [HR] = 1.49, 95% CI: 0.95 to 2.45), angina (HR = 1.26, 95% CI: 0.88 to 2.16) and myocardial infarction (HR = 1.26, 95% CI: 0.72 to 2.21) when compared to LABA users.

The first methodological concern is that the study restricted the LABA group to patients prescribed with single-ingredient LABAs, but it ignored the fixed combination therapies of LABA and ICS. Since asthmatics were included in the study cohort, the single-ingredient LABAs could be dangerous to those patients. Authors could have included the

fixed combination therapies into the LABA group and adjusted for the ICS use during the analysis.

Second, it is not clear how the exposure to study medication was measured over time. The authors defined exposure as the duration of prescribed therapy plus 30 days, however, switching or adding a different long-acting bronchodilator would terminate study participating (stated in Results section first paragraph). If an outcome event of interest occurred after a treatment switch, it is not clear if the person time under the new treatment would be measured and contributed to the corresponding new exposure category. If the authors failed to do so, it could result in misclassification of exposure status, and overestimate the person time under the exposure to the previous treatment prior to switching, thus bias the estimates of effect.

Additionally, it appears the authors were interested in measuring the total number of outcome events, regardless of whether they were the first or later occurrences, as the authors stated that "each endpoint was analysed separately, so patients who experienced more than one end point under study were included in analyses of each event". However, the recurrent events may have a different set of causes than the first occurrence, affect subsequent exposure and thus introduce bias in the analysis.

Gershon A, et al (2013)

In 2013, Gershon and colleagues (98) published results from their study titled "Cardiovascular safety of inhaled long-acting bronchodilators in individuals with chronic

obstructive pulmonary disease". This study employed a nested case-control approach using health administrative database from Ontario to evaluate the association between use of LABAs and tiotropium and the risk of hospitalization and emergency department visits for cardiovascular events. They concluded that subjects who were newly prescribed LABAs and tiotropium suffered an increased risk of a cardiovascular event when compared to non-use of these medications [adjusted odds ratios, 1.31 (95% CI: 1.12 to 1.52) and 1.14 (95% CI: 1.01 to 1.28), respectively]. However, no significant differences in cardiovascular events were found between the two types of long-acting bronchodilators. The study may have methodological limitations that could have biased their findings.

First, the increased risk of cardiovascular events seen with LABAs and tiotropium when compared to nonusers (odd ratios: 1.31 and 1.14, respectively) may be due to the noticeable imbalance between cases and controls in COPD characteristics in the time period prior to cohort entry. Table 2.5 is adapted from Gershon et al.'s Table 3 of COPD Characteristics.

Table 2.5 Imbalance between cases and controls in COPD characteristics

	% of Subjects	
	Cases	**Controls**
COPD Characteristics		
Specialist visit in previous year	74.8	71.8
ED visit for COPD		
In-past 6 mo	5.1	3.4
>6 mo before index date	13.4	11.7
Never	81.5	84.9
ED visit for acute respiratory disease		
In-past 6 mo	3.3	2.3
>6 mo before index date	14.6	12.4
Never	82.1	85.3

ED, emergency department.

As seen in the summarized table, participants in cases group had more emergency department visits for COPD and respiratory diseases than those in controls group prior to cohort entry, which indicates that participants in cases group may have had more severe COPD and were more likely to have an exacerbation of COPD symptom, an important risk factor of a cardiovascular event. The authors did not explicitly indicate that the study was sufficiently controlled for the timing of COPD exacerbations relative to the time of exposure of long-acting bronchodilators.

Second, the study may also have subjected to protopathic bias, a bias that occurs when a pharmaceutical agent is prescribed for an early manifestation of a disease that has not yet been diagnosed. The authors used a 90-day time period to define LABA and tiotropium exposure in COPD, which may have consisted of a mix of exposures that could be categorized as both new use and past use. Therefore, a more refined analysis, one that can account for the timing of exposure in LABA and tiotropium patients, may further explore the true estimate of effect.

Third, it is not clear whether the study endpoint is the date of first hospitalization, or if patients were followed through March 31, 2009. Therefore, the inclusion of periods of hospitalization may lead to immeasurable time bias. The medication dispensed during hospitalization was not available in the study dataset, thus, the exposure status of LABAs and tiotropium would not have been known during the periods of hospitalization. This underestimation of exposure will be more likely to impact cases than controls, which results in an underestimated estimate of effect.

Finally, the study did not consider the non-hospital deaths among all participants or patients newly prescribed with LABAs and tiotropium. Since it is not known how it would be different according to exposure status, the impact on the point estimate is unknown.

Wilchesky M, et al (2008)

Wilchesky M et al (99) published a study entitled "Bronchodilator Use and the Risk of Arrhythmia in COPD. Part 2: Reassessment in the Larger Quebec Cohort". The study protocol is available in Chest 2012. A nested case-control study design was employed to assess the rate ratio of arrhythmia associated with new use of short and long-acting β_2-agonists and a short-acting muscarinic antagonist. Their analysis used conditional logistic regression and controlled for COPD disease severity, cardiovascular disease, and other comorbidities. The authors concluded that the new use of SABA (Rate Ratio [RR] = 1.27; 95% CI: 1.03 to 1.57) and LABA (RR = 1.47, 95% CI: 1.01 to 2.15) was associated with increased risk of cardiac arrhythmias, but there was a non-significant association between ipratropium and risk of arrhythmias.

The methodology of the study is thoughtful, but it is limited by the nature of the database. First, the study timeline included a cohort that initiated treatment between 1990 and 1999, and were followed until 2004. However, tiotropium was just introduced to Quebec, therefore the study was not able to include this important LAMA agent during the study.

Second, the Quebec database does not have information regarding smoking status, which is a potential important covariate when investigating the association between a respiratory medication and a cardiovascular event.

Additionally, the study investigated rate ratio of arrhythmia by the class of bronchodilator instead of each individual agents. Therefore, the study results are not usable for recommending the use of a specific bronchodilator in patients with COPD.

2.5 Background and rationale for this study

Long-acting bronchodilators are the mainstay of pharmacologic therapy for moderate to severe COPD (11), yet there is a concern regarding their cardiovascular safety. The primary effect of β_2-agonists is to dilate the bronchi by binding to the β2 receptors present in airway smooth muscle cells (39). β_2-agonists exert a physiologic effect opposite to that of β-blockers (100), which are of benefit in patients with hypertension or coronary artery disease. The potential cardiac adverse events of β_2-agonists include increase in blood pressure, tachycardia, and palpitations (101, 102). The effect of muscarinic antagonists occurs through antagonism of Ach release at M-muscarinic receptors (11). Among 5 distinct M receptors (M1-M5), the M2 receptor is located in the heart. The stimulation of M2 receptors is associated with negative chronotropic and inotropic effects, and inhibition of M2 receptors through antagonism of muscarinic receptors may cause tachycardia and myocardial ischaemia (103).

To date, clinical trials (46-75) have not assessed the association between adverse cardiovascular events and the use of long-acting bronchodilators as primary or secondary study objectives; post-hoc analyses have found that neither LABAs nor LAMA were associated with increased risk of cardiovascular events (35, 36). The results from these clinical trials may be significantly limited due to the exclusion of patients with prior cardiovascular disease. However, there are two clinical trials which included patients with prior cardiovascular disease. The Tiotropium Safety and Performance in Respimat® Trial (TIOSPIR) (67, 104) included patients with cardiovascular risk factors, but they excluded recent cardiac events within 6 to 12 months. The Study to Understand Mortality and Morbidity in COPD (SUMMIT) (105) is an on-going clinical trial focused on patients with COPD and either a history of cardiovascular disease or at increased risk for cardiovascular disease. The primary study outcome, however, is all-cause mortality in relation to the use of combined ICS and LABAs; yet the results from this study are not available. Several observational studies (98, 99) and meta-analyses (106, 107, 108) have reported adverse cardiovascular effects. However, the observed risks with long-acting bronchodilators may have been confounded by COPD exacerbations or by disease severity, and may have failed to account for protopathic and immeasurable time bias.

Importantly, neither clinical trials nor observational studies have investigated specifically whether there is an increased risk of MI among patient with COPD who are at high risk for cardiovascular disease in relation to the use of long-acting bronchodilators. Our study examined the association of long-acting bronchodilator use and the risk of MI in patients with COPD who were at high risk for cardiovascular disease.

CHAPTER 3 STUDY OBJECTIVES

3.1 Overall objective

To evaluate whether use of long-acting bronchodilators increases the risk of acute MI in patients with COPD who are at high risk for cardiovascular disease.

3.2 Primary objectives

1) To estimate the relative risk of acute MI in patients who were exposed to two long-acting bronchodilators together (LABA+LAMA or ICS/LABA+LAMA), compared to no use;
2) To estimate the relative risk of acute MI in patients who were exposed to LABA alone (LABA or ICS/LABA), compared to no use;
3) To estimate the relative risk of acute MI in patients who were exposed to LAMA alone, compared to no use.

CHAPTER 4 STUDY METHODOLOGY

4.1 Study design

A retrospective, time-matched, quasi-cohort approach (109) was applied to a new-user cohort of patients 55 years of age or older to estimate the risk of acute MI among patients with COPD who are at high risk for cardiovascular disease in relation to the use of long-acting bronchodilators.

4.2 Data source

The source of data was the Clinical Practice Research Datalink (CPRD), the world's largest database of anonymized, longitudinal primary care medical records. The database is managed by the Department of Health, UK. Approximately 13 million active patients recorded from approximately 650 primary care practices are enrolled in this UK database. The database contains information on medical diagnosis, prescriptions by general practitioners, patient characteristics and lifestyle factors (e.g., smoking). Read codes are the standard clinical terminology used in the CPRD. The CPRD data have been widely validated for COPD and cardiovascular disease (110, 111), and have been used extensively for studies of drug safety (112).

The study also used Hospital Episode Statistics (HES) database which contains details on admission, outpatient appointments and Accident and Emergency attendances at National Health Service (NHS) hospitals in England. The HES database was linked to CPRD data to form a subset of cohort for a sensitivity analysis with respect to immeasurable time bias (113).

4.3 Study population

The *source cohort* included patients 55 years of age or older with at least one newly prescribed LABA (salmeterol or formoterol), or LAMA (tiotropium), or combination therapy of ICS/LABA (fluticasone/salmeterol, budesonide/formoterol, beclometasone/formoterol, fluticasone/formoterol) from September 2003 to August 2011. The *source cohort* entry date was defined as the date of the first such prescription in this period. Patients were excluded if they had received any of these medications in the two years prior to cohort entry date, or if less than two years of "up-to-standard" medical history in the general practice.

From this *source cohort*, a *study cohort* was further restricted to patients who were at high risk for cardiovascular disease. Risk factors for cardiovascular disease used similar criteria to those developed for the SUMMIT trial (105): established coronary artery disease (CAD), established peripheral vascular disease (PVD), previous stroke, previous MI, hypertension, hypercholesterolaemia, and diabetes. Additionally, patients with end-stage chronic renal disease were excluded. Both Read codes and prescription codes, if applicable, within two years prior to the cohort entry were used to define the above conditions. Medications associated with these conditions included angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), beta blockers, anti-hypertensives, lipid lowering agents and anti-diabetics. CAD, PVD, stroke, and MI, however, were considered to be present if recorded any time prior to cohort entry.

Patients who met all the inclusion criteria were followed up for a maximum of two years from cohort entry, until an acute MI diagnosis, death from any cause, patient was no longer in the CPRD system, or end of the study period (August 2013), whichever came first.

4.4 Selection of cases and quasi-cohort person-moments

Cases were defined as patients who had an acute MI diagnosis during the follow-up period. The event date was defined as the first record of MI diagnosis in the general practice during the follow-up. Quasi-cohort person-moments (controls) for each case were randomly selected from the risk set of the cohort still at risk for an acute MI on the case's event date. For each case, up to 5 person-moments (controls) were randomly selected from the case's risk set. Quasi-cohort person-moments were matched on age (±1 year) at cohort entry, sex, cohort entry date (± 30 days), linkage eligibility to the HES database at any time during study period, and who were alive and at risk on the event date.

4.5 Exposure to long-acting bronchodilators

Current exposure was defined by prescriptions for long-acting bronchodilators received within 60 days of the event date. Long-acting bronchodilators included LABAs (formoterol or salmeterol), or LAMA (tiotropium bromide), or LABA in combination with ICS (fluticasone/salmeterol, budesonide/formoterol, beclometasone/formoterol, fluticasone/formoterol).

The exposure categories for this study included the following:

Exposure category	
Combined long-acting bronchodilators	LABA+LAMA, or ICS/LABA+LAMA
Monotherapies	LAMA alone
	LABA alone (LABA, or ICS/LABA)

The unexposed referent category (no use) was defined as no prescription of any of the above drug classes during the entire 60-day period leading up to the event date.

4.6 Covariates

The following comorbid conditions identified *at any time prior to cohort entry* were included in the multi-level regression model as covariates: CAD, PVD, stroke, arrhythmia, and congenital structural cardiovascular abnormalities. Other covariates were identified *within two years prior to cohort entry*: COPD, asthma, hypertension, diabetes, hypercholesterolaemia, renal failure, and cancer (exclude non-melanoma skin cancer). In order to identify these comorbidities, both Read codes and prescriptions for medications associated with these conditions, if applicable, within two years prior to cohort entry were obtained. These medications included ACE inhibitors, ARBs, beta-blockers, thiazide diuretics, loop diuretics, anti-arrhythmics, anti-hypertensives, lipid lowering agents, anti-diabetics, digoxin (digitalis), nitrates, aspirin, insulin, and nonsteroidal anti-inflammatory drugs (NSAIDs). Other covariates included COPD exacerbations, smoking status, and use of other respiratory medications (e.g., SABAs, ipratropium, methylxanthines, or ICS) within two years prior to the cohort entry date.

Furthermore, two stratified analyses were carried out. Exacerbations of COPD were identified within 60-day time period prior to the event date since it is possible that the risk of MI is particularly elevated among subjects with a recent or ongoing COPD exacerbation. COPD exacerbation was defined by Read codes or a new prescription of prednisolone (no prednisolone in the last 90 days). Prescription of an ICS within the 60-day time period prior to event date was identified since this may modify the association between the use of long acting bronchodilators and acute MI. Stratified analyses were carried out among subjects with and without a COPD exacerbation and with or without a concurrent ICS prescription.

4.7 Data analysis

For a time-matched, quasi-cohort approach, crude quasi-rates were calculated for each exposure category using person-moments from the quasi-cohort and corresponding sampling frame from the person-moments of the full cohort (109). The formula used to compute quasi-rate of outcome per person-moment is:

$$Quasi - rate = (n/N)(x_1/n_1)$$

Where n is person-moments of the selected quasi-cohort; N is person-moments of the full cohort; x_1 is outcome events in the exposed group; and n_1 is matched quasi-cohort person-moments for the exposed group (109).

Conditional logistic regression was used to estimate the adjusted quasi-rate ratios of MI with regards to different long-acting bronchodilator exposures in patients with COPD who were at high risk for cardiovascular disease. The calculated rate ratio provides an

accurate estimate of the incidence rate ratio of acute MI associated with the use of long-acting bronchodilators.

Finally, the crude and adjusted quasi-rate differences were computed using the Approximate Multiplicative Method (109). The formula used to compute quasi-rate difference is:

$$Quasi - rate\ difference = R_t(RR_1 - 1)/(P_0 + \sum P_k RR_k)$$

Where R_t is the overall rate of the outcome event from the full cohort; RR_k is the estimated adjusted rate ratio for exposure category k relative to the reference (k =1 to c), P_k and P_0 denote the prevalence of exposure for the different categories and the reference, respectively, $(P_0 + \sum P_k) = 1$, estimated from the quasi-cohort (109).

All analyses were performed with SAS version 9.4.

4.8 Sensitivity analyses

Three sensitivity analyses were considered in this study. First, prescriptions for any long-acting bronchodilators within 15 days of event date were excluded. Exposure status was then defined by prescription for long-acting bronchodilators of study interest during the 16-75 day time period prior to the event date. The rational for this sensitivity analysis was to minimize potential protopathic bias. A previous study showed that there was a sevenfold increase in the risk of MI in patients with an initial prescription of an inhaled β_2-agonist (95) within 3 months prior to their event date.

Second, there is a concern of potential depletion of susceptibles when evaluating the effect of current use of the long-acting bronchodilators versus non-current use, that is, a decreasing risk of an MI after an initial period of increased risk. Therefore, the identified MI cases were stratified according to whether they occur within 60 days after cohort entry or later to test for depletion of susceptibles.

Third, there is a concern of immeasurable time bias from hospitalization due to misclassification of exposure status during a hospitalization. Therefore, we performed a sensitivity analysis to exclude any patients who were hospitalized within 90-day period prior to the event date.

4.9 Sample size and power

It was estimated that there would be a cohort of 35,770 patients with COPD based on a previous study that investigated COPD and the risk of cardiovascular disease (114).This study also reported a relative risk of developing an incident diagnosis of MI (OR=1.4, 95% CI: 1.13 to 1.73) in patients with COPD as compared to COPD-free controls. If we assume 60% of patients with COPD were exposed to at least one long-acting bronchodilator, a total of 42,000 person-years of observation are expected with the maximum follow-up of two years. The rate of MI was expected to be approximately 20 per 1000 per year (115, 116). Based on aforementioned, we anticipated a relative rate of 1.20 with 97% power. This estimate was within the ranges of those studies reporting odd ratios of MI in patients with COPD (1.14-1.52) (98, 117).

4.10 Ethics approval

This study, among a series of population-based observational studies, was granted ethics approval by both Independent Scientific Advisory (ISAC) committee for Medicines and Healthcare products Regulatory (MHRA) database research for using anonymized CPRD data and HES data (13_093RA) and by Research Ethics Board of Jewish General Hospital in Montreal, Canada (13_096).

CHAPTER 5 RESULTS

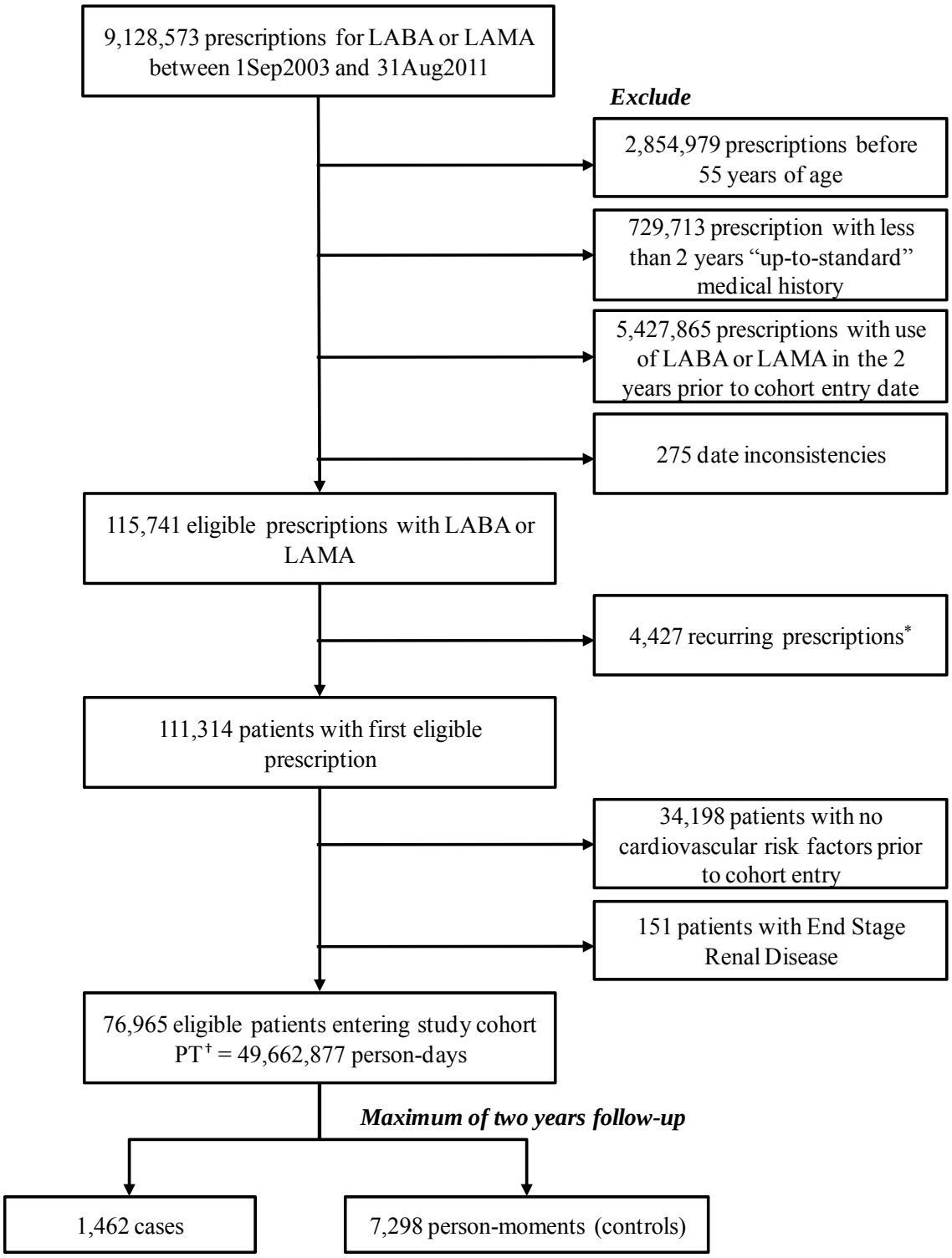
5.1 Preface

The study objectives were met by conducting a study using a quasi-cohort approach within a source population identified from CPRD. Section 5.2 describes the source population. Sections 5.3 - 5.7 present the primary results of the study. Comparisons of the primary results are made by presenting them using quasi-cohort and traditional nested case-control approaches. Section 5.8 addresses results for sensitivity analyses.

5.2 Source population

A total of 76,965 subjects who were newly prescribed at least one long-acting bronchodilator from September 2003 to August 2011, and who were at high risk for cardiovascular disease at cohort entry, were identified using the CPRD and followed up from the first prescription up to a maximum of two years. The cohort contributed more than 49.6 million person-days of follow-up. Figure 5.1 shows derivation of the study population.

Figure 5.1 Derivation of the study population



* There were patients who had more than one eligible prescription: they used a LABA or LAMA, stopped for at least 2 years and reinitiated. Since we only study the first time use of LABA or LAMA, 4,427 recurring prescriptions were excluded.

† PT, person time.

5.3 Sample size of the quasi-cohort study population

Of 76,965 eligible subjects, 1, 462 subjects had the outcome event of acute MI during more than 49.6 million person-days of follow-up. The overall incidence rate of acute MI was 10.8 per 1000 person-years. A total of 7,298 person-moments (five-to-one), matched to these cases, were selected by incidence density random sampling from the 49.6 million person-days of follow-up.

5.4 Baseline characteristics of selected quasi-cohort

Among the selected quasi-cohort of 7,298 person-moments, 2,404 were in subjects with no prescription of any long-acting bronchodilator during the 60-day period leading to the event date, 782 were in subjects with combination use of LABA and LAMA together (LABA/LAMA), 3,095 were in subjects prescribed a LABA alone, and 1,017 were in subjects prescribed a LAMA alone. Table 5.1 describes subject characteristics by the four exposure categories in the selected quasi-cohort sample.

A majority of subjects were male (ranging from 61.2% for users of LABA alone to 71.2% for users of LABA/LAMA combination). The mean age was approximately 74 years (ranging from 74.0 for no use to 75.2 years for users of LAMA alone). The prevalence of CAD, PVD, stroke, previous MI, COPD, and renal disease at the time of cohort entry was higher in the LABA/LAMA combination and LAMA alone groups than in the no use and LABA alone groups. Concomitant medications taken at the time of cohort entry were fairly balanced among the four exposure categories, although there were more subjects taking aspirin in the LABA/LAMA combination (50.6%) and LAMA

alone groups (55.5%) than those in the no use (46.9%) and LAMA alone (46.9%) groups. Furthermore, there were more ex-smokers and current smokers in the LABA/LAMA combination and LAMA alone groups (89.0% and 86.6%) when compared to the no use and LAMA alone groups (69.5% and 68.8%).

Table 5.1 Baseline characteristics of the quasi-cohort of 7,298 person-moments selected by incidence density random sampling from the 49.6 million person-days of follow-up generated by the cohort of 76,965 subjects identified from the CPRD during 2003-2011, by exposure to current use of long-acting bronchodilators

	Long-acting bronchodilator use*			
	No use†	**LABA/LAMA**	**LABA alone**	**LAMA alone**
No. person moments	2,404	782	3,095	1,017
Age (yrs); mean (SD)	74.0 (9.4)	74.1(8.7)	74.4 (9.1)	75.2 (8.6)
Male sex; %	62.4	71.2	61.2	65.4
Comorbidities at the time of cohort entry; %				
Coronary artery disease	41.3	42.8	40.8	42.8
Peripheral vascular disease	12.2	18.4	11.8	14.1
Stroke	9.2	9.6	9.0	12.3
Arrhythmia	15.7	12.8	14.4	16.6
Congenital structural cardiovascular abnormalities	0.1	0.0	0.3	0.4
Previous MI	13.4	16.8	12.5	15.5
COPD	37.5	70.6	41.0	72.6
Asthma	35.4	25.6	47.6	19.6
Hypertension	21.1	20.7	22.5	21.8
Renal disease	10.5	11.3	9.1	11.4
Cancer (excluding non-melanoma skin cancer)	3.7	3.3	2.8	4.5
Other medications taken at the time of cohort entry; %				
ACE inhibitors	41.1	45.1	43.0	43.6
ARBs	17.9	12.8	19.1	16.0
Beta-blockers	23.5	23.3	21.1	27.4
Thiazide diuretics	30.7	27.8	32.9	27.3
Loop diuretics	32.7	36.6	32.7	35.2
Other anti-hypertensives	43.9	47.4	47.4	44.4
Anti-arrhythmics	8.9	8.3	10.4	8.2

Lipid lowering agents	55.5	55.8	53.0	59.2
Anti-diabetics	13.6	11.5	12.3	13.5
Digoxin (digitalis)	6.9	7.5	7.2	7.5
Nitrates	14.2	17.3	15.4	16.5
Aspirin	46.9	50.6	46.9	55.5
NSAIDs	32.1	26.9	28.6	24.3
Use of other respiratory medication at the time of cohort entry; %				
SABAs	77.2	86.3	89.1	81.2
Ipratropium	20.8	40.8	26.7	32.5
Methylxanthines	2.9	4.9	3.0	2.6
ICS	45.1	44.4	60.2	36.1
Smoking; %				
Ex-smokers/smokers	69.5	89.0	68.8	86.6

* No use refers to no prescription of any long-acting bronchodilator during the 60-day period leading up to the event date.
LABA/LAMA use refers to the combination use of LABA and LAMA, therefore, at least one prescription of LABA and one prescription of LAMA during the 60-day period prior to the event date.
LABA alone use refers to at least one prescription of LABA only during the 60-day period prior to the event date.
LAMA alone use refers to at least one prescription of LAMA only during the 60-day period prior to the event date.

† Reference category.

5.5 Baseline characteristics of matching cases and controls

Table 5.2 provides a comparison of subject characteristics between cases and controls using the traditional nested case-control approach. As expected, cases appear to be "sicker" and were receiving more medications for cardiovascular disease than controls. Case subjects were more likely to have had CVD, PVD, stroke, arrhythmia, congenital structural cardiovascular abnormalities, previous MI, COPD, renal disease, and cancer, and more likely to have been using ACE inhibitors, ARBs, beta-blockers, loop diuretics, other anti-hypertensive agents, lipid lowering agents, anti-diabetics, digoxin, nitrates, aspirin, and ipratropium at cohort entry. More cases than controls were ex-smokers and

current smokers (cases: 77.6% vs. controls: 73.6%). These differences were adjusted for in the analysis.

Table 5.2 Baseline characteristics of cases of acute MI and controls

	Cases (N=1,462)	Controls (N=7,298)
Years of follow up from cohort entry to index date*; mean (SD)	0.9 (0.6)	0.9 (0.3)
Age (yrs)*; mean (SD)	74.4 (9.2)	74.4 (4.1)
Male sex*; %	63.3	63.3
Comorbidities at the time of cohort entry; %		
Coronary artery disease	61.2	41.4
Peripheral vascular disease	19.2	13.0
Stroke	11.6	9.6
Arrhythmia	16.4	15.0
Congenital structural cardiovascular abnormalities	0.6	0.2
Previous MI	31.8	13.6
COPD	52.1	47.4
Asthma	31.9	37.3
Hypertension	21.8	21.7
Renal disease	15.5	10.2
Cancer (excluding non-melanoma skin cancer)	4.8	3.4
Other medications taken at the time of cohort entry; %		
ACE inhibitors	51.2	42.7
ARBs	19.0	17.6
Beta-blockers	32.4	23.0
Thiazide diuretics	28.3	30.8
Loop diuretics	47.5	33.5
Other anti-hypertensives	53.7	45.8
Anti-arrhythmics	9.9	9.4
Lipid lowering agents	65.5	54.9
Anti-diabetics	19.6	12.8
Digoxin (digitalis)	8.1	7.2
Nitrates	31.5	15.4
Aspirin	61.4	48.5
NSAIDs	29.7	28.9

Use of other respiratory medication at cohort entry; %		
SABAs	81.7	83.8
Ipratropium	31.9	27.1
Methylxanthines	3.2	3.1
ICS	45.8	50.1
Smoking; %		
Ex-smokers/smokers	77.6	73.6

* Matching variables.

5.6 Quasi-rates, crude and adjusted rate ratios and rate differences of acute MI

Table 5.3 presents the numbers of events and selected quasi-cohort person-moments, as well as the corresponding quasi-rates, rate ratios and rate differences for current use of long-acting bronchodilators relative to no use (reference group) during the 60-day period prior to the event date using the quasi-cohort approach.

Of the 1,462 events of acute MI, 486 occurred in the no use group, 185 in users of the LABA/LAMA combination group, 585 in the LABA alone group and 206 in the LAMA alone group. The computed quasi-rates were 10.85, 12.70, 10.15, and 10.87 per 1,000 person-years for no use, LABA/LAMA combination, LABA alone and LAMA alone, respectively. The adjusted quasi-rate ratios of the LABA/LAMA combination, LABA alone and LAMA alone were 1.06 (95% CI: 0.82 to 1.37), 1.04 (95% CI: 0.85 to 1.27), and 0.91 (95% CI: 0.74 to 1.11), relative to no current use. In all instances, the adjusted rate ratios were close to unity, and no statistically significant associations between the use of long-acting bronchodilators and acute MI were observed.

When the analysis was further stratified by prescription of an ICS within the 60-day time period prior to event date, the sub-cohorts with and without a concurrent ICS prescription

were fairly balanced. A total of 676 events of acute MI, matched with 3,248 person-moments were observed in the sub-cohort without a concurrent ICS prescription; while a total of 786 cases, matched with 4,050 person-moments were observed in the sub-cohort with a concurrent ICS prescription.

For the sub-cohort without a concurrent ICS prescription, the adjusted quasi-rate ratios of LABA/LAMA combination, LABA alone, and LAMA alone were 1.46 (95% CI: 0.74 to 2.89), 0.81 (95% CI: 0.54 to 1.22) and 0.99 (95% CI: 0.75 to 1.30), relative to no current use. These adjusted quasi-rate ratios can be converted to approximate adjusted rate differences of 2.30 (95% CI: -1.48 to 8.29), -0.95 (95% CI: -2.58 to 0.97), and -0.07 (95% CI: -1.39 to 1.30) per 1,000 person-years, relative to no current use. For the sub-cohort with a concurrent ICS prescription, the adjusted quasi-rate ratios of LABA/LAMA combination, LABA alone, and LAMA alone were 1.02 (95% CI: 0.68 to 1.55), 1.01 (95% CI: 0.70 to 1.46), and 0.97 (95% CI: 0.57 to 1.66), relative to no current use. The adjusted rate differences were computed as 0.13 (95% CI: -2.62 to 2.18), 0.07 (95% CI: -2.42 to 1.85) and -0.15 (95% CI: -3.47 to 2.62) per 1,000 person-years when compared to no current use. All 95% CIs for all exposure categories crossed the null; no statistically significant associations were observed between use of long-acting bronchodilators and acute MI when stratifying the cohort by concurrent ICS prescription during the 60-day period prior to the event date.

The analysis was further stratified by COPD exacerbation occurring during the 60-day time period prior to the event date. A total of 1,304 events of acute MI, matched with

6,857 person-moments were observed in the sub-cohort without a COPD exacerbation. For this sub-cohort, the adjusted quasi-rate ratios of LABA/LAMA combination, LABA alone, and LAMA alone were 1.11 (95% CI: 0.84 to 1.45), 1.10 (95% CI: 0.89 to 1.37), and 0.88 (95% CI: 0.71 to 1.09), relative to no current use. The approximate adjusted rate differences were computed as 0.99 (95% CI: -1.68 to 3.58), 0.96 (95% CI: -1.19 to 2.94), and -1.11 (95% CI: -3.06 to 0.68), when compared to no current use. Again, all 95% CIs crossed the null, no statistically significant associations between current use of long-acting bronchodilators and acute MI were found in the sub-cohort of subjects without COPD exacerbation.

There were only 158 events of acute MI observed in the sub-cohort with COPD exacerbation. A total of 441 person-moments were matched to the cases. The adjusted quasi-rate ratios cannot be computed in this instance because this stratification lead to many strata containing no exposed or no non-exposed subjects. Therefore, the covariates CAD, congenital structural cardiovascular abnormalities, cancer, thiazide diuretics, anti-arrhythmics, SABAs and smoking had to be excluded from the logistic regression. The adjusted quasi-rate ratios of LABA/LAMA combination, LABA alone, and LAMA alone were 1.45 (95% CI: 0.05 to 46.33), 0.58 (95% CI: 0.04 to 8.59), and 0.15 (95% CI: 0.00 to 6.69), relative to no current use. These adjusted quasi-rate ratios were converted to approximate adjusted rate differences of 0.69 (95% CI: -4.92 to 3.99), -0.65 (95% CI: -4.96 to 0.67) and -1.31 (95% CI: -5.14 to 0.50) per 1,000 person-years, relative to no current use. All 95% CIs were fairly wide and crossed the null, therefore, no statistically

significant associations were observed between the use of long-acting bronchodilators and acute MI in the sub-cohort of subjects with COPD exacerbation.

Table 5.3 Quasi-rates and crude and adjusted rate differences of acute MI with use of long-acting bronchodilators

Quasi-cohort size: five-fold	No. with acute MI	No. quasi-cohort person-days	Quasi-rates (per 1,000 person-years)*	Crude quasi-rate ratio	Adjusted quasi-rate ratio †	Crude quasi-rate differences (per 1,000 person-years)	Adjusted quasi-rate differences (per 1,000 person-years)
Long-acting bronchodilators use							
Number	1,462	7,298					
No use‡	486	2,404	10.85	1.00	1.00 (reference)	0 (reference)	0 (reference)
LABA/LAMA	185	782	12.70	1.17	1.06 (0.82 - 1.37)	1.85	0.65 (-2.15 - 3.37)
LABA alone	585	3,095	10.15	0.94	1.04 (0.85 - 1.27)	-0.71	0.37 (-1.88 - 2.45)
LAMA alone	206	1,017	10.87	1.00	0.91 (0.74 - 1.11)	0.02	-0.99 (-3.13 - 0.98)
Stratify by prescription of an ICS within the 60-day period prior to event date							
Sub-cohort of subjects without a concurrent ICS prescription							
Number	676	3,248					
No use	434	2,068	5.01	1.00	1.00 (reference)	0 (reference)	0 (reference)
LABA/LAMA	18	66	6.51	1.30	1.46 (0.74 - 2.88)	1.50	2.30 (-1.48 - 8.29)
LABA alone	59	336	4.19	0.84	0.81 (0.54 - 1.22)	-0.82	-0.95 (-2.58 - 0.97)
LAMA alone	165	778	5.07	1.01	0.99 (0.75 - 1.30)	0.05	-0.07 (-1.39 - 1.30)
Sub-cohort of subjects with a concurrent ICS prescription							
Number	786	4,050					
No use	52	336	4.61	1.00	1.00 (reference)	0 (reference)	0 (reference)
LABA/LAMA	167	716	6.95	1.51	1.02 (0.68 - 1.55)	2.34	0.13 (-2.62 - 2.18)
LABA alone	526	2,759	5.68	1.23	1.01 (0.70 - 1.46)	1.07	0.07 (-2.42 - 1.85)
LAMA alone	41	239	5.11	1.11	0.97 (0.57 - 1.66)	0.50	-0.15 (-3.47 - 2.62)
Stratify by COPD exacerbation occurring during the 60-day period prior to event date							
Sub-cohort of subjects without COPD exacerbation							

Number	1,304	6,857					
No use	452	2,317	9.84	1.00	1.00 (reference)	0 (reference)	0 (reference)
LABA/LAMA	154	708	10.97	1.12	1.11 (0.84 - 1.45)	1.13	0.99 (-1.68 - 3.58)
LABA alone	517	2,867	9.09	0.92	1.10 (0.89 - 1.37)	-0.74	0.96 (-1.19 - 2.94)
LAMA alone	181	965	9.46	0.96	0.88 (0.71 - 1.09)	-0.38	-1.11 (-3.06 - 0.68)
Sub-cohort of subjects with COPD exacerbation [§]							
Number	158	441					
No use	34	87	1.27	1.00	1.00 (reference)	0 (reference)	0 (reference)
LABA/LAMA	31	74	1.36	1.07	1.45 (0.05 - 46.33)	0.09	0.69 (-4.92 - 3.99)
LABA alone	68	228	0.97	0.76	0.58 (0.04 - 8.59)	-0.30	-0.65 (-4.96 - 0.67)
LAMA alone	25	52	1.56	1.23	0.15 (0.00 - 6.69)	0.29	-1.31 (-5.14 - 0.50)

[*] Quasi-rates computed using person-moments from quasi-cohort and corresponding sampling fraction from the 49.7 million person-days of the full cohort.

[†] Adjusted quasi-rate ratios were computed by logistic regression.

[‡] Reference category.

[§] Covariates coronary artery disease, congenital structural cardiovascular abnormalities, cancer, thiazide diuretics, anti-arrhythmics, SABAs and smoking were from the logistic regression.

5.7 Rate ratios of acute MI using nested case-control approach

Table 5.4 presents the crude and adjusted rate ratios of acute MI associated with each of the four long-acting bronchodilator categories during the 60-day period before the event date using the nested case-control approach. All adjusted rate ratios are close to unity and not significant.

Table 5.4 Crude and adjusted rate ratios of acute MI associated with use of long-acting bronchodilators as compared with no current use during the 60-day period prior to event date

Independent exposure categories	N(%) of cases exposed	N (%) of controls exposed	Crude rate ratio (95% CI)	Adjusted rate ratio (95% CI)
Long-acting bronchodilators use				
No use	486 (33.24)	2,404 (32.94)	1.00 (reference)	1.00 (reference)
LABA/LAMA	185 (12.65)	782 (10.72)	1.17 (0.97 - 1.41)	1.06 (0.82 - 1.37)
LABA alone	585 (40.01)	3,095 (42.41)	0.93 (0.81 - 1.07)	1.04 (0.85 - 1.27)
LAMA alone	206 (14.09)	1,017 (13.94)	1.00 (0.83 - 1.20)	0.91 (0.74 - 1.11)
Stratify by prescription of an ICS within the 60-day period prior to event date				
Sub-cohort of subjects without a concurrent ICS prescription				
No use	434 (64.20)	2,068 (63.67)	1.00 (reference)	1.00 (reference)
LABA/LAMA	18 (2.66)	66 (2.03)	1.44 (0.78 - 2.67)	1.46 (0.74 - 2.89)
LABA alone	59 (8.73)	336 (10.34)	0.79 (0.55 - 1.15)	0.81 (0.54 - 1.22)
LAMA alone	165 (24.41)	778 (23.95)	0.99 (0.78 - 1.25)	0.99 (0.75 - 1.30)
Sub-cohort of subjects with a concurrent ICS prescription				
No use	52 (6.62)	336 (8.30)	1.00 (reference)	1.00 (reference)
LABA/LAMA	167 (21.25)	716 (17.68)	1.51 (1.04 - 2.21)	1.02 (0.68 - 1.55)
LABA alone	526 (66.92)	2,759 (68.12)	1.18 (0.84 - 1.67)	1.01 (0.70 - 1.46)
LAMA alone	41 (5.22)	239 (5.90)	1.15 (0.71 - 1.87)	0.97 (0.57 - 1.66)
Stratify by COPD exacerbation occurring during the 60-day period prior to event date				
Sub-cohort of subjects without a COPD exacerbation				
No use	452 (34.66)	2,317 (33.79)	1.00 (reference)	1.00 (reference)
LABA/LAMA	154 (11.81)	708 (10.33)	1.12 (0.91 - 1.37)	1.11 (0.84 - 1.45)
LABA alone	517 (39.65)	2,867 (41.81)	0.91 (0.79 - 1.06)	1.10 (0.89 - 1.37)
LAMA alone	181 (13.88)	965 (14.07)	0.94 (0.77 - 1.14)	0.88 (0.71 - 1.09)

Sub-cohort of subjects with a COPD exacerbation*				
No use	34 (21.52)	87 (19.73)	1.00 (reference)	1.00 (reference)
LABA/LAMA	31 (19.62)	74 (16.78)	1.61 (0.36 - 7.23)	1.45 (0.05 - 46.33)
LABA alone	68 (43.04)	228 (51.70)	1.29 (0.40 - 4.24)	0.58 (0.04 - 8.59)
LAMA alone	25 (15.82)	52 (11.79)	1.25 (0.27 - 5.81)	0.15 (0.00 - 6.69)

* Covariates coronary artery disease, congenital structural cardiovascular abnormalities, cancer, thiazide diuretics, anti-arrhythmics, SABAs and smoking were from the logistic regression.

5.8 Sensitivity analyses

5.8.1 Sensitivity analysis pertaining to protopathic bias

Protopathic bias occurs when a drug is prescribed for an early manifestation of a disease that has not yet been diagnosed (118). In this study, the problem of protopathic bias may have occurred in situations when a subject presented with breathlessness and chest pain, yet a diagnosis of angina was missed and possibly mistaken as COPD exacerbation, and the subject was prescribed a long-acting bronchodilator medication. Angina is an important risk factor for acute MI; when an acute MI is later diagnosed, a causal relationship may be incorrectly inferred between the use of long-acting bronchodilators and acute MI. To address this issue, prescriptions for any long-acting bronchodilator within 15 days of event date were excluded. Instead, the exposure status was defined by a prescription during the 16-75 day time period prior to the event date. Tables 5.5 and 5.6 present the results of this sensitivity analysis using the quasi-cohort and nested case-control approaches.

From the sensitivity analysis pertaining to protopathic bias, of the 1,462 events of acute MI, 523 occurred in the no use group, 178 in users of the LABA/LAMA combination group, 566 in the LABA alone group and 195 in the LAMA alone group. The adjusted quasi-rate ratios of LABA/LAMA combination, LABA alone and LAMA alone were

1.16 (95% CI: 0.92 to 1.46), 1.12 (95% CI: 0.94 to 1.34) and 0.97 (95% CI: 0.79 to 1.19), relative to no current use. These adjusted quasi-rate ratios can be converted to approximate adjusted rate differences of 1.60 (95% CI: 0.96 to 6.03), 1.24 (95% CI: -0.70 to 4.43), and -0.28 (95% CI: -2.36 to 2.47) per 1,000 person-years, relative to no current use. All 95% CIs crossed null in all exposure categories. Similar to the primary results above, this sensitivity analysis shows that the use of long-acting bronchodilators does not increase the risk of acute MI in subjects with COPD at high risk for cardiovascular disease.

Table 5.5 Sensitivity analysis pertaining to protopathic bias using quasi-cohort approach

Quasi-cohort size: five-fold	No. with acute MI	No. quasi-cohort person-days	Quasi-rates (per 1,000 person-years)	Crude quasi-rate ratio	Adjusted quasi-rate ratio	Crude quasi-rate differences (per 1,000 person-years)	Adjusted quasi-rate differences (per 1,000 person-years)
Long-acting bronchodilators use*							
Number	1,462	7,298					
No use	523	2,674	10.50	1.00	1.00 (reference)	0 (reference)	0 (reference)
LABA/LAMA	178	740	12.91	1.23	1.16 (0.92 - 1.46)	0.23	1.60 (-0.96 - 6.03)
LABA alone	566	2,928	10.38	0.99	1.12 (0.94 - 1.34)	-0.01	1.24 (-0.70 - 4.43)
LAMA alone	195	956	10.95	1.04	0.97 (0.79 - 1.19)	0.04	-0.28 (-2.36 - 2.47)

* The exposure status was defined by prescription during the 16-75 day time period prior to event date.

Table 5.6 Sensitivity analysis pertaining to protopathic bias using nested case-control approach

Independent exposure categories	N(%) of cases exposed	N (%) of controls exposed	Crude rate ratio (95% CI)	Adjusted rate ratio (95% CI)
Long-acting bronchodilators use*				
Number	1,462	7,298		
No use	523 (35.77)	2,674 (36.64)	1.00 (reference)	1.00 (reference)
LABA/LAMA	178 (12.18)	740 (10.14)	1.23 (1.02 - 1.50)	1.16 (0.92 - 1.46)
LABA alone	566 (38.71)	2,928 (40.12)	0.99 (0.86 - 1.14)	1.12 (0.94 - 1.34)
LAMA alone	195 (13.34)	956 (13.10)	1.04 (0.87 - 1.26)	0.97 (0.79 - 1.19)

* The exposure status was defined by prescription during the 16-75 day time period prior to event date.

5.8.2 Sensitivity analysis pertaining to depletion of susceptibles

Depletion of susceptibles occurs when evaluating the effect of current use of a medication versus non-current use (94). Generally, the risk associated with use of a therapeutic agent does not remain constant over time. Patients who remain on an agent are likely to be those who can tolerate it better than those who are susceptible (119, 120). In this study, the problem of depletion of susceptibles may have occurred in subjects who had an outcome event of acute MI 60-day after cohort entry or later. To address this issue, the acute MI cases were stratified according to whether they occurred within 60 days after cohort entry or later. The reference category was changed from no use to LAMA alone group since all patients were prescribed with a long-acting bronchodilator at cohort entry. Table 5.7 presents results of this sensitivity analysis using the nested case-control approach. All the rate ratios were close to unity, and no significant associations between the use of long-acting bronchodilators and acute MI were found.

Table 5.7 Sensitivity analysis pertaining to depletion of susceptibles

Independent exposure categories	N(%) of cases exposed	N (%) of controls exposed	Crude rate ratio (95% CI)	Adjusted rate ratio (95% CI)
Acute MI occurred within 60 days after cohort entry				
LAMA alone*	46 (23.23)	213 (21.60)	1.00 (reference)	1.00 (reference)
LABA/LAMA	27 (13.64)	62 (6.29)	2.01 (1.15 - 3.51)	1.55 (0.75 - 3.19)
LABA alone	125 (63.13)	711 (72.11)	0.80 (0.55 - 1.17)	0.73 (0.44 - 1.23)
Acute MI occurred after 60 days after cohort entry				
LAMA alone*	160 (12.66)	804 (12.74)	1.00 (reference)	1.00 (reference)
No use	486 (38.45)	2,404 (38.09)	1.01 (0.83 - 1.23)	1.12 (0.91 - 1.39)
LABA/LAMA	158 (12.50)	720 (11.41)	1.10 (0.87 - 1.41)	1.19 (0.89 - 1.59)
LABA alone	460 (36.39)	2,384 (37.77)	0.97 (0.80 - 1.18)	1.27 (0.99 - 1.63)

* Reference category.

5.8.3 Sensitivity analysis pertaining to immeasurable time bias

Immeasurable time bias arises from the unidentified presence of hospitalization when defining drug exposure (121). In this study, the drug exposure was defined using prescription records on an outpatient basis in CPRD. When a subject is hospitalized, during the time of hospitalization, the subject cannot be classified as being exposed to a drug or not. In order to address this issue, a sensitivity analysis was performed to exclude any subjects who were hospitalized within 90 days prior to the event date. A subset of the cohort was then generated so that cases and person-moments could be matched on linkage eligibility to the HES database at any time during the study period. Tables 5.8 and 5.9 describe the results for this sensitivity analysis.

In this sub-cohort, 411 events of acute MI were observed. A total of 3,953 person-moments, were selected to match these cases. Of the 411 events of acute MI, 127 occurred in the no use group, 50 in users of the LABA/LAMA combination group, 183 in the LABA alone group, and 51 in the LAMA alone group. The adjusted quasi-rate ratios of LABA/LAMA combination, LABA alone and LAMA alone were 1.22 (95% CI: 0.74 to 2.01), 1.20 (95% CI: 0.82 to 1.75), and 0.89 (95% CI: 0.60 to 1.33), relative to no current use. These adjusted quasi-rate ratios can be converted to approximate adjusted rate differences of 0.60 (95% CI: -0.95 to 4.54), 0.54 (95% CI: -0.66 to 3.37), and -0.30 (95% CI: -1.43 to 1.46) per 1,000 person-years, relative to no current use. All 95% CIs crossed null in all exposure categories. This sensitivity analysis shows that the use of long-acting bronchodilators does not increase the risk of acute MI in subjects with COPD at high risk for cardiovascular disease in this sub-cohort.

Table 5.8 Sensitivity analysis pertaining to immeasurable time bias using quasi-cohort approach

Quasi-cohort size: five-fold	No. with acute MI	No. quasi-cohort person-days	Quasi-rates (per 1,000 person-years)	Crude quasi-rate ratio	Adjusted quasi-rate ratio	Crude quasi-rate differences (per 1,000 person-years)	Adjusted quasi-rate differences (per 1,000 person-years)
Long-acting bronchodilators use							
Number	411	3,953					
No use	127	1,275	2.90	1.00	1.00 (reference)	0 (reference)	0 (reference)
LABA/LAMA	50	410	3.55	1.22	1.22 (0.74 - 2.01)	0.22	0.60 (-0.95 - 4.54)
LABA alone	183	1,740	3.06	1.06	1.20 (0.82 - 1.75)	0.06	0.54 (-0.66 - 3.37)
LAMA alone	51	528	2.81	0.97	0.89 (0.60 - 1.33)	-0.03	-0.30 (-1.43 - 1.46)

Table 5.9 Sensitivity analysis pertaining to immeasurable time bias using nested case-control approach

Independent exposure categories	N (%) of cases exposed	N (%) of controls exposed	Crude rate ratio (95% CI)	Adjusted rate ratio (95% CI)
Long-acting bronchodilators use				
No use	127 (30.90)	1,275 (32.25)	1.00 (reference)	1.00 (reference)
LABA/LAMA	50 (12.17)	410 (10.37)	1.28 (0.89 - 1.86)	1.22 (0.74 - 2.01)
LABA alone	183 (44.53)	1,740 (44.02)	1.11 (0.86 - 1.43)	1.20 (0.82 - 1.75)
LAMA alone	51 (12.41)	528 (13.36)	1.01 (0.70 - 1.45)	0.89 (0.60 - 1.33)

CHAPTER 6 DISCUSSION AND CONCLUSIONS

6.1 Association between use of long-acting bronchodilators and acute MI

We identified 76,965 subjects who were 55 years of age or older with newly prescribed long-acting bronchodilators from September 2003 to August 2011 using CPRD. Among them, 1,462 had the outcome event of acute MI during more than 49.6 million person-days of follow-up. The results of this observational and population-based study using the quasi-cohort approach found that the use of LABA and LAMA, given alone or together, do not increase the risk of acute MI in patients with COPD at high risk for cardiovascular disease. The results also remained robust in sensitivity analyses. This is the first study, to our knowledge, that evaluates the risk of acute MI and use of long-acting bronchodilators in a cohort of patients with COPD who are also at high risk for cardiovascular disease.

Our results are consistent with the post-hoc analyses of the 3-year TORCH trial (12236), 4-year UPLIFT trial (6835), and a systematic review (123) of clinical trial safety databases for tiotropium that, all concluded that long-acting bronchodilators do not increase the risk of cardiovascular events in patients with COPD. The results are also consistent with the most recently published retrospective analysis of the UPLIFT trial. Tashkin et al (2015) (124) performed Kaplan-Meier analyses on patients who experienced a cardiac event and remained in UPLIFT. The results of the analyses revealed a later occurrence of cardiac severe adverse events with tiotropium HandiHaler® when compared with placebo.

Our results also agree with a previous observational study conducted by de Luise and colleagues (125). The authors conducted a cohort study in 10,603 patients who were 40 years of age or older at the time of the COPD hospitalization from 1977 to 2003 using the Danish healthcare registries. Identified patients were followed up for a period of 18-24 months until a hospitalization with a cardiac discharge diagnosis of MI, congestive heart failure, angina, atrial fibrillation or atrial flutter, supraventricular tachycardia, or ventricular arrhythmia. The study observed an incidence rate ratio of 1.25 (95% CI: 0.49 to 3.17) for the occurrence of MI, suggesting that there is no statistically significant association between risk of MI and use of tiotropium relative to no use.

Our results, however, contradict those of previous observational studies that reported increased risk among patients with COPD taking LABAs or LAMAs relative to no use of either drug. Those studies, however, may have a number of limitations that could have biased their findings and explained their divergent results. Gershon and colleagues (98) conducted a nested case-control study using the health administrative database from Ontario and found that newly prescribed LABAs and tiotropium increased the risk of a cardiovascular event. The increased risk of cardiovascular events with LABAs and tiotropium when compared to non users may be due to the noticeable imbalance between cases and controls in COPD characteristics in the time period prior to cohort entry. The authors did not explicitly indicate that the study was sufficiently adjusted for the timing of COPD exacerbations relative to the time of exposure of long-acting bronchodilators. Second, the study failed to assess potential protopathic bias. The authors used a 90-day time period to define LABA and tiotropium exposure, which may have consisted of a mix

of exposure that could be categorized as both new use and past use. Therefore, the study should have adjusted for this or, as our study did, perform a sensitivity analysis, to re-define exposure status by excluding prescriptions 15-day leading to the event date. Third, the inclusion of period of hospitalization may lead to immeasurable time bias, as the exposure status cannot be identified during the periods of hospitalization. Finally, the study did not consider the non-hospital deaths among patients newly prescribed with LABAs and tiotropium. Since it is not known how it would be different according to exposure status, the impact on the point estimate is unknown.

Furthermore, Verhamme et al 2012 (93) and Jara et al 2008 (97) investigated the association between the use of long-acting bronchodilators and risk of adverse cardiovascular events. As a result, both concluded that the current use of tiotropium was not associated with an increased risk of adverse cardiovascular events when compared to the current use of LABA. However, with absence of no use group, the results cannot imply any association between the use of LABA and LAMA and cardiovascular safety. Other limitations of these two studies were illustrated in chapter two section 2.4.1.

6.2 Strengths and Limitations

There are several strengths to this study. This is the first study to investigate the safety of long-acting bronchodilators in a cohort of patients with COPD who are at high risk for cardiovascular disease. Previous clinical trials focused on investigating the benefits of long-acting bronchodilators in terms of improvement of lung function, reduction of COPD exacerbations, and health-related quality of life. Although post-hoc analyses of

those clinical trials tried to explore the cardiac safety among long-acting bronchodilator uses, those studies may be significantly limited due to the exclusion of patients with prior cardiovascular disease. The SUMMIT trial (105), an on-going clinical trial, focused on patients with COPD and either a history of cardiovascular disease or at increased risk for cardiovascular disease. However, the results from the SUMMIT trial are not available. Additionally, the sample size was limited in previous meta-analysis (123) of randomized trials for tiotropium, only 212 cases arose out of a cohort of 19,545 subjects. However, we were able to achieve an adequate power to investigate the association between the use of long-acting bronchodilators and risk of MI in patients with COPD. Our study stems from a large cohort of 76,965 subjects with more than 49.6 million person-days of follow-up, out of which 1,462 outcome events arose. Several observational studies assessed the association between use of long-acting bronchodilators and risk of MI, however, none of them explored if it is safe to prescribe long-acting bronchodilators to COPD patients who are already at high risk for cardiovascular disease. Finally, we are able to conclude with confidence that long-acting bronchodilators are safe to be prescribed to patients with COPD and who are at high risk for cardiovascular disease. The adjusted quasi-rate ratios of the LABA/LAMA combination, LABA alone and LAMA alone were 1.06 (95% CI: 0.82 to 1.37), 1.04 (95% CI: 0.85 to 1.27), and 0.91 (95% CI: 0.74 to 1.11), relative to no current use. In all instances, the adjusted rate ratios were close to unity, and no statistically significant associations between the use of long-acting bronchodilators and acute MI were observed. Even at worst, from the upper limit of 95% CIs, we would observe a 37% increase of acute MI in users of the LABA/LAMA

combination group, 27% increase in the LABA alone group and 11% increase in the LAMA alone group, relative to no current use.

Our study is the first study to apply the quasi-cohort approach which upgrades the nested case-control method. The quasi-cohort approach provides the clinically relevant comparison of patient characteristics according to treatment group rather than comparing cases expected to be sicker, with controls. In the nested case-control studies, subject characteristics were presented as risk factors for the outcome, while presentation of quasi-cohort data focuses on describing the underlying cohort, illustrating any imbalance in subject characteristics by exposure status (109). Additionally, the quasi-cohort approach can measure the effect of drug exposure on the outcome by rate differences, which is more intuitive to understand than rate ratios.

Furthermore, our study used CPRD which contains information for important lifestyle factors such as smoking status. The study was therefore able to adjust for smoking, which is an important risk factor for both acute MI and COPD.

Lastly, protopathic bias, depletion of susceptibles and immeasurable time bias were considered in the sensitivity analyses.

Our study also has some limitations. First, when the analysis was further stratified by COPD exacerbation during the 60-day time period prior to the event date, only 158 events of acute MI, matched with 441 person-moments, were observed in the sub-cohort

of subjects with COPD exacerbation. Therefore adjustment for potential confounders was less complete and power was limited for this important sub-group analysis.

Second, like all other observational studies using medical databases, although there is complete documentation on prescription, patients' behavior in taking these medications, such as whether patients adhere to the prescribed medications, is unknown.

Third, although we tried our best to include only patients with COPD, there is a likelihood of subjects with asthma in the study cohort, especially in the users of LABA alone group. Diagnosis of asthma was much higher in the LABA alone group compared with the other groups. The prevalence of cardiovascular diseases and COPD was much lower in users of the LABA alone group than those of the LABA/LAMA combination and LAMA alone groups. Additionally, more subjects in the LABA alone group were taking inhaled corticosteroids. These are all signs showing there are asthmatic subjects in the study cohort. However, our study adjusted for diagnosis of COPD and asthma at cohort entry.

Fourth, there is still potential for unmeasured confounding variables. The unmeasured confounding variables can include lifestyle factors, such as lack of physical activity, stress, and illegal drug use, which are other risk factors for cardiovascular disease. Despite these potential limitations, our study offers some evidence that initiation of treatment with long-acting bronchodilators does not increase the risk of acute MI in patients with COPD at high risk for cardiovascular disease. The clinical implication is

that it should be safe for clinicians to give an initial long-acting bronchodilator to patients with COPD who are also at high risk for cardiovascular disease.

6.3 Areas for future research

Future research into evaluating whether the use of long-acting bronchodilators increases the risk of acute MI in patients with COPD who are at high risk for cardiovascular disease should conduct a study with adequate power to look at risk in patients with concomitant COPD exacerbation. To achieve that, the study may select more quasi-cohort person-moments (controls) to match with the cases to increase the sample size in the cohort of patients with concomitant COPD exacerbation.

Future research could also examine the risk of acute MI associated with new long-acting bronchodilators. There are now novel long-acting bronchodilators approved to treat patients with COPD. These newly licensed long-acting bronchodilators include once-daily LABAs, indacaterol, olodaterol, vilanterol, and once daily LAMA glycopyrronium, and twice-daily LAMA aclidinium bromide. It would also be important to conduct future research to investigate the association between the use of long-acting bronchodilators and other comorbidities (e.g., hospitalizations for community acquired pneumonia, angina, and stroke).

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