

WP2 Framework for pharmacoepidemiological studies

WG1 Databases

Study Protocol

**Use of inhaled long acting beta2 adrenoceptor
agonists and the risk for Acute Myocardial Infarction
(AMI)**

**A methodological comparison across data sources and
epidemiological design**

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WG1 Drug AE group

Name	Role
Marietta Rottenkolber ¹	Protocol lead
Sven Schmiedl ³	Protocol backup
Arlene Gallagher ⁴	Protocol reviewer
Xavier Kurz ⁵	Protocol reviewer
Omer de Mol ⁶	Protocol reviewer
Saga Johanson ⁷	Protocol reviewer
Frans Rutten ²	Protocol reviewer
Kourtney Davis ⁸	Protocol reviewer
Ana Sofia Afonso	Protocol reviewer
Marietta Rottenkolber ¹	Database 1 (Bavaria) lead
Joerg Hasford ¹	Database 1 (Bavaria) backup
Miguel Gil ⁹	Database 2 (Bifap) lead
Consuelo Huerta ⁹	Database 2 (Bifap) backup
Ulrik Hesse ¹⁰	Database 3 (DKMA) lead
Frank de Vries ²	Database 3 (DKMA) backup
Paola Primatesta ¹¹	Database 4 (GPRD) lead
Dan Dedman /Jenny Campbell ⁴	Database 4 (GPRD) backup
Eef Voogd ² ,Olaf Klungel ²	Database 5 (Mondriaan) lead
Liset van Dijk ²	Database 5 (Mondriaan) backup
Yolanda Alvarez ⁵	Database 6 (THIN) lead
Ana Ruigomez ¹²	Database 6 (THIN) backup
Mark de Groot ² and Raymond Schlienger ¹¹	WG1 colead
Olaf Klungel ² and Robert Reynolds ¹³	WP2 coleads

1 Institute for Medical Information Sciences, Biometry and Epidemiology, Ludwig-Maximilians-Universitaet Munich, Germany (LMU Muenchen)

2 Universiteit Utrecht, Utrecht, The Netherlands (UU)

3 Philipp Klee-Institute for Clinical Pharmacology, HELIOS Clinic Wuppertal, Wuppertal, Germany / Department of Clinical Pharmacology, School of Medicine, Faculty of Health, Witten/Herdecke University, Germany

4 General Practice Research Database, London, United Kingdom (GPRD)

5 European Medicines Agency, London, United Kingdom (EMA)

6 Genzyme Europe B.V., The Netherlands

7 AstraZeneca AB, Sweden (AZ)

8 GlaxoSmithKline Research and Development LTD, Belgium (GSK)

9 Agencia Espanola de Medicamentos y Productos Sanitarios, Madrid, Spain (AEMPS)

10 Lægemedelstyrelsen (Danish Medicines Agency), Copenhagen, Denmark (DKMA)

11 Novartis Pharma AG, Basel, Switzerland (Novartis)

12 Fundación Centro Español de Investigación Farmacoepidemiológica, Madrid, Spain (CEIFE)

13 Pfizer Ltd, New York, USA (Pfizer)

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1 Context of the studies

The studies described in this protocol are all performed within the framework of PROTECT (Pharmacoepidemiological Research on Outcomes of Therapeutics by a European Consortium) Workpackage 2 (WP2) and Workgroup 1. Primary aim of WP2 is to develop, test and disseminate methodological standards for the design, conduct and analysis of Pharmacoepidemiological (PE) studies applicable to different safety issues and using different data sources. Workgroup 1 will evaluate results from PE studies on 5 key adverse events (AEs) related to specific exposure(s) of interest, performed in different databases. Therefore, emphasis will be on evaluating the methodological aspects of the studies in this protocol and not on the clinical consequences of the association under investigation. The standards to be developed by WP2 will contribute to decrease the discrepancies in results from different studies in the future and increase the usefulness, validity, and reliability of these studies for benefit-risk assessment in the EU.

2 Background

2.1 Introduction

Asthma and chronic obstructive pulmonary disease (COPD) are the most common chronic airway diseases in the western world. For both, a stepwise treatment to reduce symptoms, improve lung function, and prevent risk of exacerbation is recommended using several drug classes according to guidelines published by e.g. the Global Initiative for Asthma [GINA guideline] (1) and the Global Initiative for Chronic Obstructive Lung Disease [GOLD guideline] (2), respectively. Beta-2-adrenoceptor agonists (B2A) are therapeutic mainstays in treating asthma and COPD due to their bronchodilative effects mediated by B2A. This drug class consists of two types of drugs: short acting B2A (SABA) which are used as a reliever medication and long acting B2A (LABA) which are used as maintenance / controller medication. Widely used SABA compounds with a half-life of a few hours only are salbutamol, fenoterol and terbutalin.

Formoterol and salmeterol are the most frequently used LABA compounds with a half-life between 5-15 hrs and therefore, these compounds most commonly have labelled indications for use twice a day. Interestingly, onset of bronchodilative effect differs significantly between LABA compounds: after using formoterol relevant effects occurs after less than 5 minutes whereas 2 hours were revealed for salmeterol (overview see (3)). Therefore, a third term (RABA "rapid acting B2A") has been introduced in several guidelines (e.g. GINA (1)) including most SABA's and the long-acting compound formoterol whereas in other guidelines, RABA has not been introduced (British Thoracic Society guideline on the management of asthma (4)).

A second bronchodilative drug class acts on the cholinergic system (muscarinic antagonists [MA]) and as for B2A, drugs can be divided according to their half-life in short acting MA (SAMA) and long acting (LAMA). Commercially available compounds are ipratropium and tiotropium.

Taking into account differences in pathophysiological aspects between asthma and COPD, the indication and therefore usage of B2A and MA differs between both diseases. In asthma patients, B2A (SABA and LABA) are the preferred bronchodilative compounds according to the guidelines and MA are more used in combination with B2A if additional bronchodilation is required. On the other hand, usage of MA and B2A are each recommended starting in Step 2 of the GOLD guidelines as options for long-acting bronchodilation for COPD patients. Therefore, the fractions of patients treated with B2A (SABA, LABA) and MA (SAMA and LAMA) is expected to differ between patient groups.

Focussing on cardiac side effects of B2A one must consider that drugs with an opposite mechanism of action (beta-adrenoceptor-antagonists) have well-known cardio protective effects and are widely used in patients suffering from e.g. ischemic heart disease, hypertension and acute myocardial infarction (AMI)). Conversely, stimulation of cardiac beta-adrenoceptors as done by B2A may have deleterious cardiovascular effects particularly in patients with cardiac risk factors (5). And in fact, tachycardia and arrhythmias are well-known side effects of B2A confirming a cardiac influence of these drugs particularly after oral therapy (due to a high systemic exposure) as stated in the respective summary of product characteristics (SPCs), e.g. clenbuterol

(Spiropent(R)). Obviously, inhaled drugs cause much smaller systemic exposure but cardiac side effects (e.g. arrhythmias, tachycardia) are also described in the respective SPCs (e.g. formoterol [Foradil(R)]. Furthermore, cardiac side were also reported after exposure with inhaled MA (e.g. ipratropium [Atrovent(R)]).

Several observational studies have been performed on the association between the usage of inhaled B2A and the occurrence of AMI (6-8). However, these studies have produced conflicting results. Reasons for this variation are numerous, e.g. small number of events (AMI) leading to poor precision of risk estimate, potential misclassification of potential cardiac events versus airway-related events due to similar clinical complaints, differences in populations of drug users, measurement of drug exposure, and background risk of AMI. Additionally, a consensus document was released in 2000, redefining AMI (9).

In the following section, an overview of relevant randomised controlled trials (RCT), meta-analyses and observational studies will be given focussing on adverse events with particular emphasis on cardiac-related events.

2.2 Randomized Controlled Trials

2.2.1 Asthma

Since several systematic reviews examining the efficacy and the safety of LABA in asthmatic patients have been performed recently by the Cochrane Collaboration (see below), a systematic review in terms of repeating these analyses in this protocol would be redundant. Instead, relevant RCTs will be discussed briefly in the following section prior to describing the results of respective meta-analysis.

There are several studies evaluating the efficacy of LABA in patients suffering from asthma. Unfortunately, side effects are only reported in some of these studies in a more or less structured way (see table 1). Interestingly, in none of these studies AMI has been reported as a (serious) adverse event ((S)AE) but some of the reported AE's (chest tightness, chest pain) could be associated with an ischemic cardiovascular event. On the other hand, these symptoms might also be associated with a lacking efficacy in treating asthma and therefore a clear discrimination between cardiac- or airway-relatedness cannot be made. Thus (and due to the small number of reported AEs in large RCTs [see table 1]) a final statement regarding the risk for AMI in asthmatic patients using LABA is still missing.

A large pragmatic randomized trial evaluating the safety of salmeterol compared to placebo added to standard care in asthma (10) (SMART-study [Salmeterol Multicenter Asthma Research Trial]), reported an unexpected increase in African Americans in the primary endpoint "combined respiratory-related deaths or life-threatening experiences" (RR=4.1 [95% CI: 1.5-10.9]) at an interim analysis. These observed results of elevated risk in a subgroup treated with salmeterol and difficulties in enrolment led to a premature study termination by the sponsor. The discussion section stated that lower usage of corticosteroids and lower peak expiratory flow (at screening) in African Americans compared to Caucasian might be related to the increased risk as well as some other factors (e.g. genetic predisposition, patients' behaviour). There was also a (non-significant) increase in all secondary endpoints in Caucasian patients, e.g. "all-cause death" (RR=1.3 [95% CI: 0.8-2.3]), "respiratory related death" (RR=2.3 [95% CI: 0.9-5.6]) and "asthma-related death" (RR=5.8 [95% CI: 0.7-48.4]). Results of the SMART study, and in particular the impact of LABA use without concomitant inhaled corticosteroids, were widely discussed in the context of safety data from all observational and clinical trial data across LABA manufacturers and regulatory authorities of many countries took actions to increase the benefit-risk profile in asthma by limiting the use of LABA mono-therapy and maximizing its concomitant use with inhaled corticosteroids per e.g. the GINA (1), the NIH's EPR3 (11) or the British asthma treatment guidelines (4).

Interestingly, in a post-hoc analysis of the SMART study stratifying the patients with respect to their ICS-use, a (non-significant) increase in "all-cause deaths" and "respiratory-related deaths" could also be revealed in Caucasian and African American patients receiving ICS. Therefore (and due to some other reasons), the debate on the risk-benefit ratio of LABA in patients suffering from asthma is still ongoing and most recently, the FDA has required the manufacturers of LABAs to conduct four randomized, double-blind, controlled clinical trials in adolescents and adults comparing the addition of LABAs to inhaled corticosteroids versus inhaled

corticosteroids alone to evaluate the endpoint of severe asthma-related events (composite of asthma exacerbation requiring oral corticosteroids, asthma hospitalization, or asthma-related death) (12).

Table 1: Selection of large randomized controlled trials evaluating LABA in patients with asthma

Study	Accepted co-medication of interest (ICS, SABA, LABA)	Intervention	Treatment Duration	(Serious) adverse events of particular interest	Conclusion regarding particular (S)AE
Kemp et al., 1998 (13)	SABA (Albuterol) as needed and regular ICS usage	- Salmeterol 42 µg BID [S84] (n=252) or - Placebo [P] (n=254)	12 weeks	AE leading to study discontinuation: - S84: n=7; chest tightness (n=1) - P: n=5; chest pain, shortness of breath (each n=1)	No relevant differences between treatment groups
Kelsen et al., 1999 (14)	SABA (Albuterol) as needed and regular ICS usage	- Salmeterol 42µg plus beclomethasone 168 µg BID [S84/B336] (n=239) - Beclomethasone 336 µg BID [B672] (n=244)	24 weeks	AE leading to study discontinuation: - S84/B336: n=7; ventricular tachycardia, heart block - B672: n=6; palpitations / tachyarrhythmia, chest symptoms, abnormal arteriogram finding	No relevant differences between treatment groups
O'Byrne et al., 2001 (OPTIMA trial) (15)	SABA as needed	i.) Group A (no corticosteroid for ≥ 3 months, FEV1 ≥ 80% after terbutaline) - Budesonide 100 µg BID (n=228) - Budesonide 100 µg / formoterol 4.5 µg BID (n=231) - Placebo BID (n=239) ii.) Group B (≤ 400 µg/d inhaled budesonide for ≥ 3 months, FEV1 ≥ 70% after terbutaline) - Budesonide 100 µg BID (n=322) - Budesonide 100 µg plus formoterol 4.5 µg BID (n=323) - Budesonide 200 µg BID (n=312) - Budesonide 200 µg plus 4.5 µg formoterol BID (n=315)	12 months	No explicit statement (< 2% class-specific effects of beta-agonists and inhaled corticosteroids in all treatment arms)	No relevant differences between treatment groups

O`Byrne et al., 2005 (16)	None (LABA, ICS and reliever medication are given according to randomisation)	<ul style="list-style-type: none"> - Formoterol 4.5 µg / budesonide 80 µg BID plus reliever medication formoterol 4.5 µg / budesonide 80 µg given as needed [F9/B160//needed:FB4.5/B80] (n=925) - Formoterol 4.5 µg / budesonide 80 µg BID plus reliever medication terbutaline 0.4 mg given as needed; [F9/B160//needed:T0.4] (n=909) - Budesonide 320 µg BID plus reliever medication terbutaline 0.4 mg given as needed [B640//needed:T0.4] (n=926) 	12 months	<ul style="list-style-type: none"> i.) Patients with at least 1 SAE <ul style="list-style-type: none"> - F9/B160//needed:FB4.5/B80: 5 % - F9/B160//needed:T0.4: 7% - B640//needed:T0.4: 5% ii.) Cardiovascular events leading to discontinuation: <ul style="list-style-type: none"> - F9/B160//needed:FB4.5/B80: n=1 - F9/B160//needed:T0.4: n=2 - B640//needed:T0.4: n=3 <p>(General cardiovascular disorders, heart rate, and rhythm disorders, and myocardial, endocardial, and pericardial disorders and valve disorders)</p>	No relevant differences between the treatment groups
Ind et al., 2003 (17)	SABA (Salbutamol)	<ul style="list-style-type: none"> - Salmeterol 50µg +fluticasone 250µg BID [S100/F500] (n= 171) - Fluticasone 250 µg BID [F500] (n=160) - Fluticasone 500 µg BID [F1000] (n=165) 	24 weeks	<p>AE leading to study discontinuation:</p> <ul style="list-style-type: none"> S100/F500: 7 patients F500: 2 patients F1000: 6 patients <p>No explicit statement regarding affected organ classes</p>	No relevant differences between the treatment groups
Nelson et al., 2006 (SMART trial) (10)	"usual care" including ICS and SABA	<ul style="list-style-type: none"> - Salmeterol 42 µg BID [S84] (n=13,176) - Placebo [P] (n=13,179) 	28 weeks	<p>Deaths:</p> <ul style="list-style-type: none"> - S84: Hypertensive cardiovascular disease, Atherosclerotic heart disease; Congestive heart failure (each n=1) - P: Coronary atherosclerosis (n=1) <p>(non-specified SAE: 4 % in each treatment group)</p>	<p>Preliminary study termination due to unexpected findings probably related to under-usage of ICS (see above). Comparable SAE rate were revealed for both treatment groups.</p>

Focussing on safety aspects, several reviews were published by the Cochrane collaboration for both LABA compounds salmeterol and formoterol:

i.) Salmeterol

In a review of the Cochrane Collaboration focussing on serious adverse events in asthma patients, 26 trials comparing salmeterol to placebo and 8 trials comparing salmeterol to the SABA compound salbutamol were analysed representing 62,815 patients (including 2,599 children) in total (18). Concomitant use of ICS was allowed but might not be a part of the randomized treatment regime. Compared to patients receiving placebo, regular treatment with salmeterol was associated with a non-significant increase in all-cause mortality (Peto Odds Ratio = 1.3 [95% CI: 0.9-2.1]) and a significant increase regarding non-fatal SAE (Peto Odds Ratio = 1.2 [95% CI: 1.0-1.3]). Comparing patients receiving salbutamol or salmeterol, no significant increase in fatal or non-fatal SAEs could be revealed. By combining the results of the SMART-study (10) (see above) and the SNS-study (RCT comparing the safety of salmeterol twice daily or salbutamol four times a day in addition to usual care (19)) a significant increase in asthma-related death were found for regular salmeterol use compared to placebo or salbutamol in patients not taking ICS, respectively (Peto Odds Ratio = 9.5 [95% CI: 1.2-73.1]). In their conclusion, the authors doubt that the use of ICS abolish the risks of regular salmeterol.

In a second review analyzing the impact of ICS, SAEs were compared for patients receiving an ICS or ICS plus Salmeterol (20). In 30 RCTs including 10,873 adult patients, 6 patients died in the salmeterol plus ICS group compared to 5 patients in the ICS-only group (Peto Odds Ratio = 1.1 [95% CI: 0.3-3.5]). No deaths were reported in children. For non-fatal SAEs, a non-significant increase could be revealed for patients receiving salmeterol plus ICS compared to patients receiving ICS only (Peto Odds Ratio = 1.2 [95% CI: 0.9-1.5]). The authors conclude that the numbers of SAEs and deaths were too small to make a final conclusion regarding the negation of the increased SAE risk associated with the use of salmeterol by adding ICS to salmeterol.

ii.) Formoterol

In an analogue step-wise manner, SAEs have been analysed in patients treated with formoterol. In patients not treated with ICS as a part of the randomized treatment regime (22 studies with 8,032 participants (21)), 3 deaths occurred in patients receiving formoterol whereas no patient died within the placebo group (not statistically significant). Non-fatal SAEs occurred more frequently in patients treated with formoterol compared to the placebo patients (Odds Ratio: 1.6 [95% CI: 1.1-2.3]). No significant increase in fatal or non-fatal SAEs was found for formoterol if the comparator was SABA (salbutamol or terbutaline).

In a Cochrane review focussing on patients with ICS (21 studies; 10,816 patients) (22) 4 patients died receiving formoterol plus ICS whereas none of the ICS-only patients died (not statistically significant). Non-fatal SAEs were very similar in both groups (Peto Odds Ratio: 1.0 [95% CI: 0.7-1.3]) for adults but a non-significant increase was revealed in children receiving formoterol (Peto Odds Ratio: 1.6 [95% CI: 0.8-3.3]). Asthma related SAEs in patients receiving formoterol occurred less frequently in adult patients (Peto Odds Ratio: 0.5 [95% CI: 0.3-1.0]) but were more common in children (Peto Odds Ratio: 1.5 [95% CI: 0.5-4.6]). Similar to salmeterol, the authors could not exclude an increased mortality in patients receiving formoterol plus ICS compared to patients receiving ICS only.

iii.) Comparison of salmeterol / formoterol

After evaluating salmeterol and formoterol separately, head-to-head studies were analysed in a two-step manner. Focussing on patients not treated with ICS as a part of the randomized treatment regime (23) four studies including 1,116 adults and 156 children were analyzed. There was only one death in an adult (unrelated to asthma) and no deaths in children. Adult patients receiving formoterol had a non-significant lower risk for non-fatal SAEs compared to patients treated with salmeterol (Peto Odds Ratio: 0.8 [95% CI: 0.5-1.3]) whereas in children an almost comparable risk was found (Peto Odds Ratio: 1.0 [95% CI: 0.1-15.3]). The authors stated

that the events were too rare and the number of patients was too small to make a final conclusion in terms of comparing the safety of both LABA compounds.

Regarding patients with inclusion of ICS in the randomised treatment regime, 8 studies (6,163 adults) were analyzed in the most recent review (24). There was one death in each treatment group (not related to asthma). Comparing both compounds, there were no significant differences in all-cause non-fatal SAE (Peto Odds Ratio: 1.1 [95% CI: 0.8-1.6]) and in asthma-related non-fatal SAE (Peto Odds Ratio: 0.7 [95% CI: 0.4-1.3]). As stated by authors, no clear decision can be made regarding the safety profiles of both LABA compounds.

In summary, there is evidence for an increased overall or asthma-related mortality in adult asthmatic patients treated with LABA, particularly in patients not using an ICS on a regular basis. Regarding salmeterol, that assumption has also been supported by a recently published meta-analysis (25). An elevated risk for cardiac / cardiovascular events cannot be excluded in these patients

2.2.2 COPD

In comparison to patients suffering from asthma, COPD patients are high-risk patients regarding ischemic events due to high prevalence of smoking and increased age. Therefore, much higher rates of (S)AEs affecting the heart are reported in the respective RCTs. A meta-analysis published in 2003 (26) stated that salmeterol (50 µg BID) did not increase the risk of cardiovascular adverse events in comparison to placebo. Furthermore, recently published results of large trials (27, 28) did not show an increased risk for ischemic (cardiovascular) events in patients treated with LABA in comparison to several other treatments including placebo. In table 2, some examples of RCTs focussing on patients with COPD receiving LABA are depicted.

One study by Wedzicha et al. (29) reported a higher risk for unwanted cardiac side effects for patients receiving the long-acting anticholinergic drug tiotropium compared to patients receiving salmeterol and fluticasone propionate. Since a protective cardiac effect of ICS in terms of reducing inflammatory processes influencing coronary artery disease cannot be excluded (see (30)), results of Wedzicha et al. (29) are of limited value for examining cardiac effects of salmeterol due to a lacking control group receiving placebo. On the other hand, use of oral corticosteroids (OCS) is associated with an increased risk for AMI in COPD patients (31, 32). Since OCS are used for treating acute exacerbations, increased AMI risk might reflect primarily a higher probability of cardiac events in these vulnerable patients instead of a causal relationship for OCS-usage but as for all non-interventional studies, no causality statement can be made.

Table 2: Examples of randomized controlled trials evaluating LABA in patients with COPD

Study	Accepted co-medication of interest (ICS, SABA, LABA)	Intervention	Treatment Duration	(Serious) adverse events of particular interest	Conclusion regarding particular (S)AE
Aaron et al., 2007 (33)	SABA (Albuterol)	- Tiotropium 18 µg QD plus placebo BID [T18/P] (n=156) - Tiotropium 18 µg QD plus salmeterol 50 µg BID [T18/S100] (n=148) - Tiotropium 18 µg plus fluticasone 500 µg / salmeterol 50µg BID [T18/F1000/S100] (n=145)	52 weeks	Myocardial infarction or acute arrhythmia: - T18/P: n=2 (1.3%) - T18/S100: n=2 (1.4%) - T18/F1000/S100: n=2 (1.4%)	No relevant differences between the treatment groups
Calverley et al, 2007 (TORCH-trial) (34)	Other medication than LABA or corticosteroids	- Salmeterol 50µg plus fluticasone 500µg BID [S100/F1000] (n=1,546) - Salmeterol 50µg BID [S100] (n=1,542) - Fluticasone 500µg BID [F1000] (n=1,551) - Placebo BID [P] (n=1,545)	3 years	Ischemic cardiovascular adverse events*: - S100/F1000: n=144 (9%) - S100: n=166 (11%) - F1000: n=167 (11%) - P: n=166 (11%)	No relevant differences between the treatment groups
Wedzicha et al., 2008 (INSPIRE trial) (29)	SABA	- Salmeterol 50 µg / fluticasone 500 µg BID plus placebo [S100/F1000/P] (n=658) - Tiotropium 18 µg QD plus placebo [T18/P] (n=665)	2 years	i.) Cardiac SAE - S100/F1000/P: n=23 (3%) - T18/P: n=34 (5%) ii.) Cardiac disorders associated with death - S100/F1000/P: n=9 (1%) - T18/P: n=19 (3%) iii.) Clinically significant ECG abnormalities: less than 2 % in both treatments	Higher rate of cardiac SAE and cardiac disorders associated with death in patients receiving tiotropium compared to salmeterol/fluticasone patients

Hanrahan et al., 2008 (28)	SABA (Albuterol), SAMA (Ipratropium)	- Arformeterol** 15µg BID [AF15BID] (n=288) - Arformeterol 25µg BID [AF25BID] (n=292) - Arformeterol 50 µg [AF50] (n=293) - Salmeterol 42µg BID [S84] (n=290) - Placebo [P] (n=293)	12 weeks	Ischemic events: - AF15BID: n=2 (0.7%) - AF25BID: n=2 (0.7%) - AF50: n=4 (1.4%) - S84: n=2 (0.7%) - P: n=4 (1.4%)	No relevant differences between the treatment groups
Vogelmeier et al., 2011 (POET-COPD) (35)	Other Medication than LABA and anticholinergics	- Tiotropium 18 µg QD plus placebo [T18P] (n=3,711) - Salmeterol 50 µg BID plus Placebo [S100/P] (n=3,673)	1 year	i.) Cardiac SAE - T18P: n=98 (2.6%) - S100P: n=85 (2.3%) ii.) Vascular SAE - T18P: n=37 (1.0%) - S100P: n=25 (0.7%)	No relevant differences between the treatment groups

* Data were published in a post hoc analysis of the TORCH study (27)

** Arformoterol: (R,R)-enantiomer of formoterol

As for asthma, there are several reviews published by the Cochrane Collaboration analyzing the risks and benefits of LABA in treating patients suffering from COPD. Most reviews focus on efficacy (36, 37) but data regarding side effects are limited. And in particular, data regarding cardiac side effects are missing and therefore, reviews will be discussed briefly.

The combination of LABA and ICS was analysed in comparison to a) placebo (38), b) ICS (39), c) long-acting beta-agonist (40) and d) the anticholinergic compound tiotropium (41).

a) In comparison to placebo (11 studies, 6427 patients (38)), the combination of ICS and LABA (pooled analysis of studies evaluating fluticasone/salmeterol or budesonide/formoterol) led to a significant reduction in the rate of exacerbations and overall-mortality but these results were dominated by the TORCH trial (34) (see above). Regarding adverse events, fewer patients receiving the combined therapy were withdrawn from the study due to adverse events.

b) In comparison to ICS, the combination in patients suffering from COPD (7 studies, 5,708 participants (39)), showed a significant higher efficacy (e.g. reduced exacerbation rate, mortality). Between the two treatment groups, there were no relevant differences regarding the adverse event profile.

c) In comparison to the use of LABA, a combined therapy with ICS (10 studies, 7,596 patients (40)) led to a significant reduction in exacerbation rate without a significant difference in mortality. The authors conclude that the superiority of a combined therapy (in terms of efficacy) has to be viewed against a higher risk for adverse events and that further data are needed.

d) In comparison to tiotropium (3 trials, no pooled analysis (41)), the INSPIRE trial (29) (see above) has been criticized for the large number of withdrawals resulting in a reduced reliability of study results.

In summary, results of large trials (and in particular recently published results of a post-hoc analysis of the TORCH trial (27)) did not show an increased risk for cardiovascular events in COPD patients receiving salmeterol. Furthermore, a protective effect regarding cardiovascular events cannot be excluded in patients receiving salmeterol plus ICS (29).

2.3 Case-Control and Cohort studies

Case control and cohort studies of the association between use of B2A agonists and the risk of AMI have demonstrated conflicting results. In some of these studies, patients were included based on drug prescriptions irrespective of the underlying airway disease. Obviously these studies are limited by a heterogeneous population including both, asthma and COPD patients differing in their cardiovascular risk profile to a high extent as described above. On the other hand, cohort studies including patients according to their airway disease are limited due to e.g. miscoding and overlap as described below (section overlap between asthma and COPD) and a wide range of control for potential confounding by disease indication/severity. Therefore, both methodological approaches have their limitations. In table 3 some examples are shown.

Table 3 Examples of cohort and case-control studies

Author	Study type / Study population	Exposure	Outcome	Result
Au et al., 2000 (42)	Case-control study (1,444 cases, 4,094 controls) patients with treated hypertension and postmenopausal women without hypertension	Inhaled B2A, that were dispensed in metered dose inhalers (MDI)	Incident myocardial infarction	Elevated estimated risk of myocardial infarction for subjects who has filled one B2A MDI prescription in the 3 month to their myocardial infarction; adjusted OR: 1.7 (95% CI: 1.1-2.6); no dose-response relationship between B2A use and risk of myocardial infarction
Au et al., 2002 (6)	Nested case-control study (413 cases, 6,050 controls) subjects who were enrolled in the Ambulatory Care Quality Improvement Project (ACQUIP) from December 1996 through May 1999	Number of long-acting and short-acting B2A MDI canisters received during the 90 days prior to the outcome	Hospital admission for AMI or unstable angina	Risk of hospitalization for AMI or unstable angina was higher for those who used inhaled B2A in the 90 days prior the hospitalization 1-2 canisters filled adjusted OR: 1.4 (95% CI: 0.9-2.2) 3-5 canisters filled adjusted OR: 1.6 (95% CI: 1.0-2.5) ≥ 6 canisters filled adjusted OR: 1.9 (95% CI: 1.2-3.0)
Suissa et al., 2003 (7)	Nested case-control (1,127 cases, 10,766 controls) COPD patients	SABA	First occurrence of AMI (fatal and non-fatal)	No significant association between SABA use and AMI. Adjusted Rate Ratio for inhaled B2A use during the year before the myocardial infarction: any use: 1.1 (95% CI: 0.9-1.2) Use during the 2 month before the myocardial infarction: any use: 1.1 (95% CI: 1.0-1.3); new use: 1.1 (95% CI: 0.7-1.8)
Zhang et al., 2009 (8)	Cohort study (507,966 patients) Patients aged 18+ years prescribed LABA or SABA and documented in the UK General Practice Research Database	SABA, LABA, inhaled corticosteroids	Pattern of hazard ratio for myocardial infarction	The pattern of risk of myocardial infarction was broadly similar between inhaled SABA, LABA and ICS. Higher risk for new user (< 3 month) (Relative risk: SABA 2.4, LABA 1.5, ICS 1.9) and long-term heavy users (13+ RX of the same asthma drug in the year before) (Relative risk: SABA 1.6, LABA 1.1, ICS 1.7)

deVries et al., 2008 (43)	Nested Case-control study (2476 cases) Antihypertensive drug user	LABA and SABA (all sorts)	First nonfatal myocardial infarction	Only patients with ischaemic heart disease with low cumulative exposure to B2A had an increased risk of myocardial infarction (adjusted OR 2.5 (95% CI: 1.6-3.8))
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3 Objectives

To make comparing results possible, this protocol gives guidelines for conducting studies in the same way in five databases and across 3 designs (cohort, nested case-control, case-cross-over) on the association between inhaled LABA use and AMI. The main focus is to evaluate the impact of study design, population and database characteristics on the association between inhaled LABA and AMI.

4 Methods

4.1 Data Source

The proposed studies will be conducted in different databases. The different databases for the studies are described below. Not all databases are suitable to perform all studies. Therefore priority of study designs are appointed to all databases, see table 4.

Table 4. Overview of studies to be performed within the association inhaled LABA-AMI and the priority per partner and design.

Study design	Data source	Priority	Partner
Descriptive	Bavaria	High	LMU MUNCHEN
Descriptive	BIFAP	High	BIFAP
Nested case control	BIFAP	Low	BIFAP
Descriptive	DKMA	High	DKMA
Cohort	DKMA	Low	DKMA
Descriptive	GPRD	High	Novartis
Cohort	GPRD	High	Novartis
Nested case control	GPRD	Low	Novartis
Descriptive	Mondriaan	High	UU
Cohort	Mondriaan	High	UU
Nested case control	Mondriaan	Low	UU
Case crossover	Mondriaan	Low	UU
Descriptive	THIN	High	EMA
Cohort	THIN	Low	EMA
Nested case control	THIN	Low	EMA
Case crossover	THIN	Low	EMA

4.1.1 Mondriaan (The Netherlands)

The Dutch Mondriaan project is a private-public collaboration funded by the Dutch TOP Institute Pharma (44). Under the umbrella of Mondriaan, the participating databases currently include: the Dutch General Practitioner (LINH) database, The Almere Health Care (ZGA) database, The General Practitioners of Utrecht (HNU) database and The Leidsche Rijn Julius Health Centre (LRJG) database. The cumulative number of persons having data in Mondriaan reached about 1.4 million comprising mainly of general practitioners (GP) data complemented by pharmacy dispensing data and linkages to survey data. The four databases within Mondriaan have different starting dates and scope of data. LINH is the Netherlands Information Network of General Practice and holds longitudinal data on morbidity, prescription, and referrals. The GPs record data on all patient contacts, including diagnoses, referrals and prescriptions. The ZGA is a GP and pharmacy database. The HNU is a GP database set up in 1995 and includes data dating till the end of 2005. The LRJG is a GP database with a linkage to additional survey records. Survey information is periodically up-dated through follow-up, including information on a wide range of health and lifestyle related variables.

4.1.2 General Practice Research Database (UK)

The General Practice Research Database (GPRD) comprises computerized medical records of GPs from 1987 onwards (45). The database contains data from over 600 practices based throughout the United Kingdom, providing information on 12.5 million patients, of which 5 million are currently active. The data covers 7 % of the population. GPs play a gatekeeper role in the UK health care system, as they are responsible for primary health care and specialist referrals. Patients are semi-permanently affiliated to a practice, which centralizes the medical information from the GPs, specialist referrals and hospitalisations. The data recorded in the GPRD include demographic information, prescription details, clinical events, preventive care provided, specialist referrals, laboratory results, hospital admissions and death. The validity of a wide range of drug exposure data is routinely tested. Practices that want to contribute data to GPRD are carefully selected and trained in the software used to record medical data. Only those practices that meet quality standards are then used for research (about 10% of the practices that send data to GPRD do not meet the quality standards). Furthermore, validation studies are conducted regularly by comparing GPRD data to written notes of general practitioners. Recent additions to the database include external record linkage to other National Health Services (NHS) datasets, such as the national Hospital Episode Statistics (with extended data on all hospitalisations) and Death Certificates, increased availability of free text information via new automated system, the possibility of genetic linkage studies, prospective data collections such as questionnaires, copies of patient-based correspondence, the conduct of multi-country studies, and performing randomization studies within the database.

4.1.3 The Health Improvement Network (UK)

The Health Improvement Network (THIN) (46) is a collaboration between two companies, In Practice Systems Ltd. (INPS), developer of Vision software used by GPs in the UK, and EPIC, provider of access to data for use in medical research. THIN data are collected during routine practice and regularly delivered to THIN. THIN data collection has started in 2003, currently contains the electronic medical records of almost 8 million patients (more than 3 million active patients) collected from over 386 general practices in the UK. THIN database consequently covers more than 5.7% of the population in the UK (UCL, Website). Patient data are arranged in four standardised (Patient, Medical, Therapy and Additional Health Data) and one linked (postcode variable indicators) files per practice. Further information is possible to obtain via the Additional Information Service (AIS) including: questionnaires completed anonymously by the patient or GP, copies of patient-based correspondence, a specified intervention (e.g. a laboratory test to confirm diagnosis) and death certificates.

4.1.4 BIFAP (Spain)

BIFAP (*Base de datos Informatizada para estudios Farmacoepidemiologicos en Atencion Primaria* – A computerised database of medical records of Primary Care) (47) is a non-profit research project operated by the Spanish Medicines Agency (AEMPS), a public agency belonging to the Spanish Department of Health, with the collaboration of the Spanish Centre for Pharmacoepidemiological Research (CEIFE). The project has started

in 2003 having the goal to achieve a pool of collaborators in the range of 1000 general practitioners and pediatricians. Currently, 1190 physicians (995 GPs and 195 pediatricians) from 9 different autonomous communities in Spain collaborate with BIFAP and send their data to BIFAP every 6 months. BIFAP database includes clinical and prescription data from around 3.1 million patients covering around 6.8% of the Spanish population. The AEMPS has renewed its funding to BIFAP for project consolidation, for validation of information included in the databases, in addition to performing epidemiological studies.

4.1.5 Bavarian Claims Database (Germany)

The Bavarian statutory health insurance physicians' association is based on accounting information of the Bavarian physicians. This German database includes a population-based data on diagnosis and medical services, covering 10.5 million people. It is a pharmacy (claims) database linked to outpatient treatment data through general practitioners and specialists. The database exists since 2001 and covers 84% of the Bavarian population excluding those with private insurance. A population-based study on asthma treatment persistence has been done using this database (48).

4.1.6 National Databases (Denmark)

The Danish registries include computerized medical records of general practitioners and all hospital contacts, medication dispensing on a pharmacy level, and causes of death for the entire population (5.5 million inhabitants). The National Bureau of Statistics keeps computerized records of income, degree of education, working status, and civil status. The Ministry of Interior keeps records of all inhabitants and their migrations and date of birth and death. The information on outcomes will come from the National Hospital Discharge Register. The National Hospital Discharge Register was founded in 1977. It covers all inpatient contacts from 1977 to 1994 and from 1995 also all outpatient visits to hospitals, outpatient clinics, and emergency rooms. Upon discharge, the physician codes the reason for the contact using the ICD system. The code used is at the discretion of the individual physician. The register has a nationwide coverage and an almost 100% capture of contacts. In general, the validity of registrations is high. The National Health Service keeps a register of all contacts to general practitioners for reimbursement purposes. The register does not contain ICD codes for the contacts but codes for the nature of the contact (regular check-up visit, routine vaccination in children).

The Danish Medicines Agency keeps a nationwide register of all drugs sold at pharmacies throughout the country from 1994 onward (National Pharmacological Database run by the Danish Medicines Agency). Any drug bought is registered with ATC code, dosage sold, and date of sale for the period January 1, 1996, to December 31, 2009. As all sales are registered to the individual who redeemed the prescription, the capture and validity are high.

All registers can be linked through the use of a person specific code (the civil person number) given to all inhabitants, and used for all of the registrations mentioned before.

4.2 Period of valid data collection

Each data source has a period of valid data collection, from the left censoring date, up to the right censoring date. This is defined as follows:

4.2.1 GPRD /THIN/Mondriaan/BIFAP

The left censoring date is the latest of the following: the date that a practice became up to research standard (not recorded in Mondriaan and probably not in BIFAP), the date that a patient enrolled into a practice or the date that a practice was enrolled into the database, whichever came latest. The right censoring date is the earliest of the following: the date a patient died, the date a patient was transferred out of the practice, the end of the database's data collection, or the date that the practice left the database. Death may not be always well

recorded in Dutch and Spanish data; alternatively we may consider right censor a patient in these databases on his latest recorded event date or the date that a practice left the database, whichever came first.

4.2.2 Bavarian Claims Database (Germany)

The left censoring date is the earliest event that is recorded for an individual patient (prescription, diagnosis or lab test). The right censoring date is the latest event that is recorded for an individual patient (prescription, diagnosis or lab test). Death is not well recorded in Bavarian data.

4.3 Overlap between Asthma & COPD

A shared common origin has been discussed for asthma and COPD by some authors whereas others stated that both diseases represent distinct conditions regarding clinical and pathophysiological aspects (overview see (49)). As stated by Gibson (50), epidemiological studies showed that in older people with obstructive airway disease, overlapping diagnoses of asthma and COPD (overlap syndrome) could be revealed in at least 50 % of these patients (and these patients are typically excluded from current trials). By using lung function parameters for defining the diagnosis "COPD", Tinkelman et al. (51) found that 51.5% of these patients had a prior diagnosis of asthma and the authors stated that a diagnostic confusion between COPD and asthma is common. On the other hand, adult asthmatic patients had a 12-fold increased risk for developing COPD compared to non-asthmatic patients (52). And of course, these uncertainties might be reflected regarding ICD-(mis)coding as stated by Schneider et al. (53) and lack of diagnostics in general clinical practice (e.g. spirometry). Taking into account all limitations described above, all analysis will be stratified for the following three patient groups:

- i.) Patients coded with asthma but no COPD
- ii.) Patients coded with COPD but no asthma
- iii.) Patients coded with asthma and COPD

5 Study designs

5.1 Descriptive study of exposure, patient and outcome

Information on the use of inhaled LABA, the indication (without BIFAP), the patient characteristics, the frequency of the outcome (first AMI within the study period), and relevant co-morbidities and co-medication (used as confounders in the cohort study) will be evaluated in each individual database. The descriptive study will be performed in two steps. In the first step, all measurements will be calculated for the whole study population. In the second step, all measurements will be separately calculated for the three patients' strata: patients with asthma only, patients with COPD only and patients with asthma and COPD.

5.1.1 Study population

The study population will consist of all patients included in the period of valid data collection. The study period is Jan 1st 2002 - Dec 31st 2009. This date is chosen because all databases are able to deliver valid data in this time frame. Also in 2000 the definition of AMI was re-defined (9). It can be assumed that from Jan 1st 2002, all AMI cases are diagnosed following the new definition.

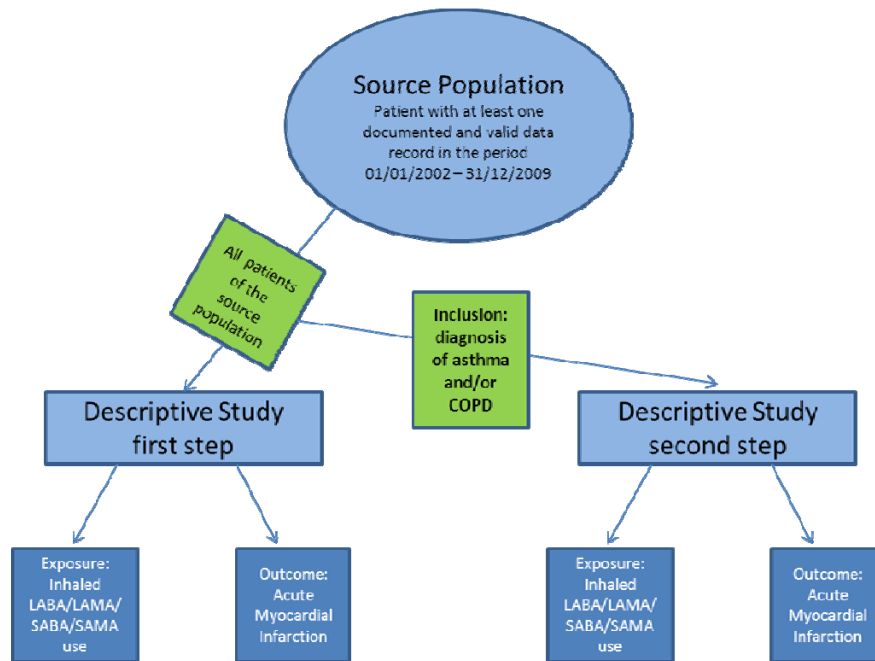


Figure 1: Study Population Descriptive Study

5.1.2 Exposure Definition (ATC codes see appendix 1)

In our analysis we will focus on the two inhaled LABA compounds, formoterol and salmeterol, without restriction of concomitant medication. Combined drugs consisting of formoterol or salmeterol will also be considered as exposure. Bambuterol, an oral LABA, will not be considered as exposure in our analysis due to an expected much smaller number of prescriptions compared to inhaled LABA and a higher systemic exposure influencing the risk for occurrence of cardiac side effects. Patients with switches between treatment groups will be counted for each group.

- Exposure: Inhaled LABA (Formoterol, Salmeterol [including combination drugs]) irrespective of other treatments
- Control group: No LABA but at least one of the following
 - o Inhaled LAMA
 - o Inhaled SAMA
 - o Inhaled SABA

The control group will be built by all patients receiving at least one Rx of LAMA, SAMA or SABA as a whole. A more detailed analysis (stratification of control drugs) will be performed in the sensitivity analysis.

5.1.3 Methods

5.1.3.1 First step

In the first step (whole study population), the following analyses should be performed

1. **Prevalence of inhaled DRUG use overall by age and sex for the 8 year period (2002-2009).** Age should be reported in ten year categories (0-9, 10-19, 20-29, 30-39, 40-49, 50-59, 60-69, 70-79, 80+). Point prevalence and period prevalence should both be reported.

a. **Point prevalence:** For each year a table including the number of inhaled DRUG Rx that start before or on June 1st and the duration includes June 1st. For each year a table including the number of DRUG Rx that start before or on March 1st and the duration includes March 1st. For each year a table including the number of DRUG Rx that start before or on September 1st and the duration includes September 1st. For each year a table including the number of DRUG Rx that start before or on December 1st and the duration includes December 1st.

b. **Period prevalence:** For each year a table including the number of inhaled DRUG Rx (only start dates are considered). Stratified by age (as stated above) and gender.

c. **Denominator**

i. First choice: Number of people present in the database at mid-year (June 1st)

ii. If first choice is not available: Number of people present in the database on January first.

iii. If first and second choice are not available: total number of people in geographically defined catchment area (if possible: for the appropriate year).

2. **Prevalence of drug use stratified by indication only for the 8 year period (2002-2009).** The indication is defined at the date of the last prescription within the study period. If the patient has only the diagnosis COPD or asthma in his medical history (considering the total study period) the patient will be included in the “COPD only” or “asthma only” stratum, respectively. Patients exhibiting a coded diagnosis of COPD **and** asthma (irrespective of whatever comes first, and irrespective of the duration between both diagnoses within the study period) will be considered as “asthma and COPD” patients. Other patients receiving exposure drugs (SABA, SAMA, LABA, LAMA) not coded with asthma or COPD within the study period will be included in “non asthma/non COPD” stratum. No stratification for age and gender will be performed.

a. **Point prevalence:** For each year a table including the number of inhaled DRUG Rx that start before or on June 1st and the duration includes June 1st by indication. For each year a table including the number of DRUG Rx that start before or on March 1st and the duration includes March 1st by indication. For each year a table including the number of DRUG Rx that start before or on September 1st and the duration includes September 1st by indication. For each year a table including the number of DRUG Rx that start before or on December 1st and the duration includes December 1st by indication.

b. **Period prevalence:** For each year a table including the number of inhaled DRUG Rx (only start dates are considered). Stratified by indication (“asthma only”, “COPD only” and “asthma and COPD” and “non asthma/non COPD”).

c. **Denominator**

i. First choice: Number of people present in the database at mid-year (June 1st)

ii. If first choice is not available: Number of people present in the database on January first.

iii. If first and second choice are not available: total number of people in geographically defined catchment area (if possible: for the appropriate year).

3. Period prevalence (per year) of inhaled DRUG ever used in that year (2002-2009) stratified by number of prescriptions (categories: 1 only, >1 & <5, 5-11, 12-23, >23).

a. **Period prevalence:** For each year a table stratified by number of Rx in that year.

b. Denominator

- i. First choice: Number of people present in the database at mid-year (June 1st)
- ii. If first choice is not available: Number of people present in the database on January first.
- iii. If first and second choice are not available: total number of people in geographically defined catchment area (if possible: for the appropriate year).

4. Prevalence of the lifetime AMI by age and sex over the first year.

The first year is the calendar year that starts after at least one year since the database came 'up to research standards. Lifetime prevalence' assessment is based on all available information for that person in the database. Real lifetime will be estimated as good as possible with the available data. Age should be reported in ten year categories (0-9, 10-19, 20-29, 30-39, 40-49, 50-59, 60-69, 70-79, 80+). Adhere to these categories, low prevalence categories will be collapsed on presentation.

a. **One table** for AMI will be computed.

b. Denominator

- i. First choice: Number of people present in the database at mid-year (June 1st)
- ii. If first choice is not available: Number of people present in the database on January first.
- iii. If first and second choice are not available: total number of people in geographically defined catchment area (if possible: for the appropriate year).

c. **First year** is the year that starts after one year of valid data collection.

d. **Age** of the patient is defined as the age at mid-year (June 1st).

5. (Cumulative) Incidence of the first AMI in database by age and gender, per year (e.g. 2003 if previous measure of prevalence was computed over 2002).

Age should be reported in ten year categories (0-9, 10-19, 20-29, 30-39, 40-49, 50-59, 60-69, 70-79, 80+). Adhere to these categories, low prevalence categories will be collapsed on presentation.

a. **Incidence** of first AMI in database. One table will be computed including the number of first AMI cases per year, by gender and age category. Calculations start the year after described in 4c (above).

b. Denominator

- i. First choice: Number of people that are presents at the start of the year (1-1 to 31-12) in the database and do not have a recorded history of AMI prior to Jan 1st of the year of interest. If a patient has more than one recorded AMI in the period from 2003 to 2009, only the first AMI considers in this calculation. After the first AMI the person is excluded from the

denominator because he is not “a person at risk” for getting his first AMI. Age is computed as age at midyear (June 1st) of the year of interest.

5.1.3.2 Second step

In the second step, measurements considered in 1.), 3.), 4.), 5.) of the first step will be separately performed for the three patients’ strata: i.) patients with asthma only, ii.) patients with COPD only and iii.) patients with COPD and asthma (if available in the database). Since patients with a diagnosis of asthma may develop COPD within the study period (and vice versa), we have to reproduce this problem in our analysis. Nevertheless, indication for each prescription is documented in some databases only and therefore, a static assessment of indication will be performed. Static assessment means that the indication is defined at the date of last prescription within the study period assessing the whole study period.

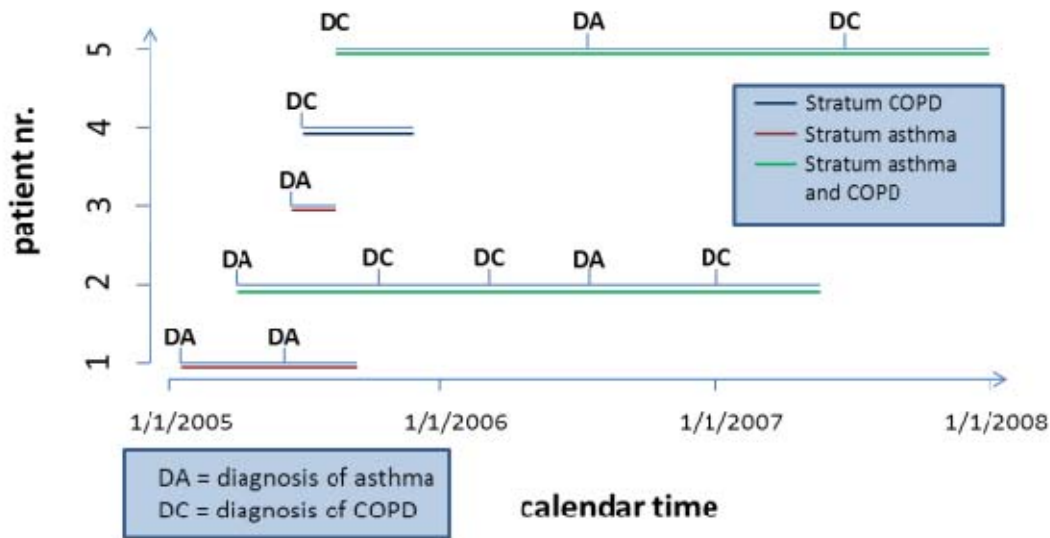


Figure 2: Change of indication: static assessment

Patients suffering neither from asthma nor from COPD will be excluded from further analysis (due to heterogeneity of diseases). (Remark: in 2.) of the first step of the descriptive study, the definition of the strata already reflects the indications. Therefore, stratification by indication for this issue is redundant.)

The denominator for the second step of the descriptive analysis is the number of patients in the respective stratum.

5.2 Cohort Study

5.2.1 Study Population

All patients, who received at least one prescription of an inhaled LABA and/or inhaled SABA and/or inhaled LAMA and/or inhaled SAMA and with coded diagnosis of asthma and/or COPD during the study period, will be included in the study. Cohort entry is the first new prescription of an inhaled B2A or an inhaled MA for the patient between January 1st 2002 and December 31st 2009 or latest data available in the database, after one year of valid data (this year can also be before January 1st 2002, when data is available).

All patients with a previous myocardial infarction will be excluded, as patients who already had a myocardial infarction have elevated risk for another AMI. For checking this exclusion criterion, longest available period documented in the respective databases (life-time period, if possible) should be analysed. All patients without a coded diagnosis of asthma or COPD will be excluded from the cohort study.

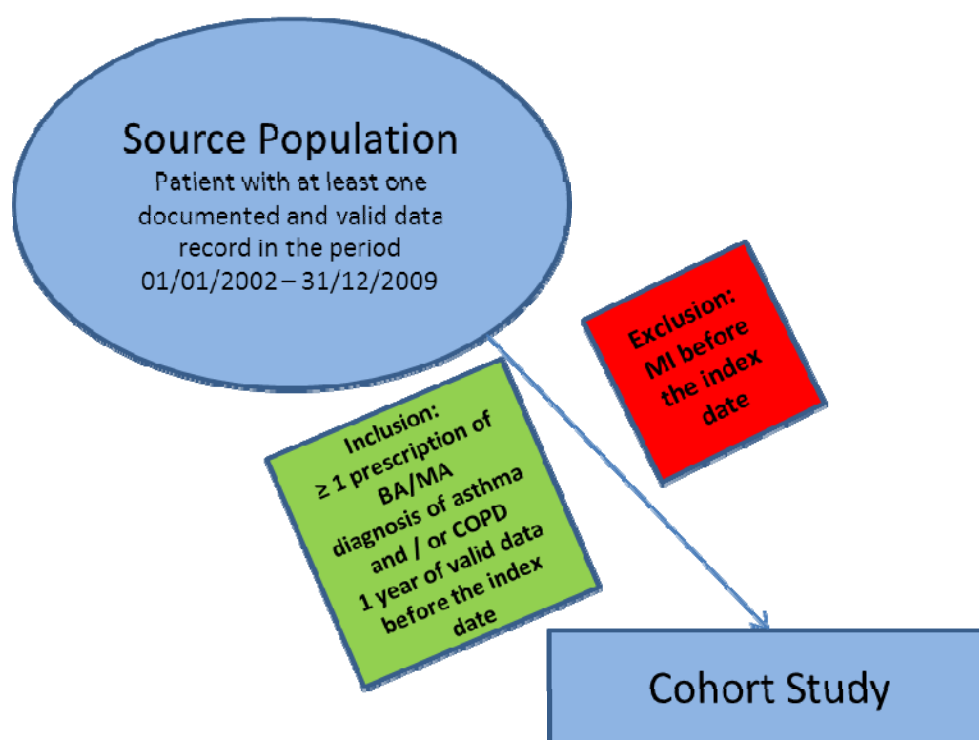


Figure 3: Study population Cohort Study

5.2.2 Definition of index date

The index date is defined for each individual patient, as the date of first prescription of an inhaled B2A or an inhaled MA after the start of valid data collection (as described above). The observation period for each patient will last from the index date to the end of data collection, the date of the first AMI in the study period, the date of death, whatever happens first.

5.2.3 Definition of exposure

The exposure of interest is inhaled LABA use. The duration of inhaled LABA use will be determined by calculating the length of treatment periods. The expected duration of each prescription/dispensing is estimated using the prescribed quantity and the prescribed daily dose. A treatment period of a patient is terminated if more than 91 days occur between the estimated end date of a prescription and the dispensing date of the next prescription. In case of missing data, e.g. the daily dose or package size for the estimation of expected duration, the age-group median duration of use of the specific database will be used. If no age-group median treatment

duration can be calculated, a treatment duration of 90 days is used as an assumption. The daily dose and the quantity supplied are often unknown in both Mondriaan databases (NPCRD and AHC), so a time period of 90 days will be used as a surrogate in both databases, due to the low reliability of the information available

If a patient has two prescriptions of the same drug class or the same drug on the same day, the respective treatment periods are summed. If the same drug class or drug is prescribed on different days, treatment duration will be calculated starting at the most recent prescription (no summation of treatment durations).

The total follow-up of the exposure to inhaled LABA will be divided into three periods.

Current user: A patient is a current user from the beginning of the prescription up to the calculated end date of the prescription.

Recent user: A patient is a recent user during the 91 days following the calculated end date of the prescription.

Past user: A patient is a past user after 91 days following the calculated end of prescription. The period of "Past user" will expire if the patient becomes a new user or on the end of follow-up.

Standard prescription durations are different for each database (and thus country). 91 days is chosen as standard in this study. Other durations are examined in a sensitivity analysis.

A patient can switch between current/recent and past periods and between the treatment classes. If a patient switches between the treatment classes a new treatment period starts at the date of the prescription of the new drug (see also chapter 3.6.3).

Current use will be stratified to the following parameters:

Duration: the duration is the sum of the consecutive periods of LABA prescription (categories 0-14 days (0-2 weeks), 14-42 days (>2 – 6 weeks), >42 days (> 6 weeks))

5.2.4 Definition of outcome

The outcome of the study is the first ever AMI in the study period. AMI is coded in the International Classification of Diseases version 10 (ICD10) as I21.- (AMI). In GPRD and THIN adequate codes read codes will be applied. In Mondriaan and BIFAP, the international classification of primary care (ICPC) code K75: AMI will be used. In sensitivity analyses we will also distinguish between fatal and non-fatal AMI for those databases that allow this distinction.

5.2.5 Change of indication (switching problem)

Since patients with a diagnosis of asthma may develop COPD within the study period (and vice versa), we have to reproduce this problem in our analysis. Nevertheless, indication for each prescription is documented in some databases only and therefore, a static assessment of indication will be performed as described in section 5.1.3.2. For defining the indication, longest available period documented in the respective databases (life-time period, if possible) should be considered. Within the sensitivity analysis, a dynamic assessment of indication will be performed. Additionally, sequence of diseases will be analysed for the COPD-and-asthma stratum (number of patients diagnosed with asthma followed by a COPD diagnosis and vice versa).

5.2.6 Confounding

In studying the safety of inhaled LABA, confounding by indication plays a major role where the disease severity determines the drug exposure. To reduce effects of confounding by indication, long term information to characterize the probability to receive inhaled LABA is essential, in order to match patients on inhaled LABA to

those on alternatives with as similar as possible clinical characteristics (e.g. CV risk factors and COPD disease severity) prior to the index prescription (55). Protopathic bias, another form of bias which occurs when the drug is prescribed for an early manifestation of a disease not yet diagnostically detected, may account for a supposedly protective effect (as in the case of Inhaled Corticosteroids (ICS)) and risk of ischemic cardiac events in patients suffering from COPD (56).

5.2.6.1 Potential confounders, for which analyses will be adjusted for, are:

- Age and sex
- Co-morbidities
- Co-medication

An adjusted analysis will be conducted with all potential confounders added to the final model. It can only be applied if as a rule of thumb there are at least 10 events per independent variable in the model. If the number of variables in the model would be too large (< 10 events per variable), selection procedure, including only potential confounders that result in a + or - 5% change of the beta-coefficient of the drug exposure of interest when the individual potential confounder is added to an age/gender adjusted model. If this still results in too many variables, only the potential confounders that change this beta-coefficient most will be included until the maximum number of variables allowed in the model is reached.

Model 1 (DSD Table 5):

Null model including age and sex.

Model 2 (Standardized analysis, possible in all databases, DSD Table 6-8):

Adjusted model

Model 1 + co-morbidities + co-medication.

Model 3 (Optimal analysis, including all covariates possible for each database, DSD Table 6-13):

Adjusted model

Model 2 + variables of the sensitivity analyses

Assessment of co-morbidities and co-medication for model 2 and 3

Assessment of co-morbidities and co-medication: every sixth months and in the case of exposure changes from one year prior to study entry until the end of the study period

Co-morbidities: from the date a patient is diagnosed with a particular disease, this disease will be considered as present disease for the whole study period onwards

Co-medication: presence of co-medication will be assessed every sixth months and a patient can switch between use and non-use in contrast to co-morbidities

5.2.7 Analysis

All analyses will be stratified as follows: "asthma only", "COPD only", "asthma and COPD" diagnosis.

In the first comparison, current users of inhaled LABA (irrespective of any other drugs) will be compared with current inhaled Non-LABA users. In the second and third analysis, current inhaled LABA users (irrespective of co-medication) will be compared with recent and past current LABA-users, respectively (irrespective of co-medication).

Definition of current Non-LABA users:

A patient is a current user from the beginning of the inhaled SABA or SAMA or LAMA prescription up to the calculated end date of the prescription (this is calculated with the prescribed daily dose or the DDD as surrogate and quantity supplied). Treatment periods will be calculated according to LABA. Obviously, the real treatment period for patients with SABA or SAMA usage is in clinical practice longer than calculated due to the irregular usage of these reliever medications. Thus, different lengths for the treatment period will be evaluated in the sensitivity analysis (180 days). After the respective treatment period, patients become (in absence of LABA usage) a non-current Non-LABA-user (no “recent” and no “past” category for Non-LABA users). If one drug defining non-LABA usage is taken as current usage then the patient will be considered as current usage irrespective of the status of the other medication.

A more detailed analysis (e.g. comparison of current LAMA- versus current LABA-users on a single compound level) will be done in the sensitivity analysis.

Switches between LABA and Non-LABA

A patient can switch between current, recent and past periods and between the treatment classes [LABA vs. Non-LABA]. If a patient switches between the treatment classes a new treatment period starts at the date of the prescription of the new drug. As described in the protocol, LABA group is defined irrespective of any other (Non-LABA) drug whereas Non-LABA group is defined by usage of SABA, SAMA or LAMA but not using LABA. So the following switches are possible:

- current LABA -> recent LABA
- recent LABA -> past LABA
- past LABA -> current LABA
- recent LABA -> current LABA
- current Non-LABA -> current LABA
- current Non-LABA -> non-current Non-LABA
- non-current Non-LABA -> current Non-LABA
- non-current Non-LABA -> current LABA

Incidence density will be calculated as the number of AMI divided by person-time. Crude incidence density ratios (IDRs) and 95% confidence intervals will be calculated by dividing the incidence density in the current users of inhaled LABA by the incidence density in the reference group (reference group 1: current inhaled Non-LABA users; reference group 2: recent inhaled LABA users, reference group 3: past inhaled LABA users). The relative risk stratified by duration of therapy and by subpopulations (i. “asthma only”, ii. “COPD only”, iii. “asthma and COPD”) will be graphically shown with the method proposed by Ramlau-Hansen for inhaled LABA and for the control group (comparisons see above) (57).

Time-dependent Cox-regression models will be used for confounding factor adjusted analysis. Co-morbidities and co-medication will be handled as time varying covariates (see 5.2.6.1). We will calculate hazard ratio for inhaled LABA compared to the control group (comparisons see above).

5.3 Nested case-control study

This study is nested in the cohort study, which was described in the previous section. The cases are the patients in the cohort study who have developed an AMI. The controls are a random selection from amongst all patients

in the cohort study who haven't had an AMI at the time when the case has had its AMI. Follow-up time, age, sex and indication will be matched for defining control patients (see below).

5.3.1 Study population

All patients of the cohort study (inhaled LABA, SABA, LAMA, SAMA user).

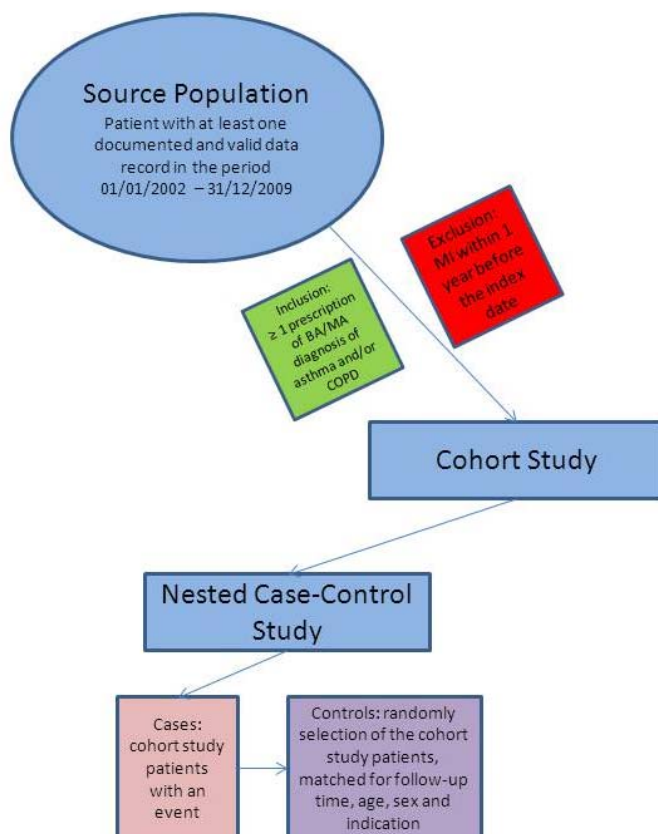


Figure 7: Study Population Nested-Case Control Study

5.3.2 Definition of cases

Cases will be all patients included in the cohort study with a diagnosis of an incident AMI. The date of the AMI will be considered the outcome day.

5.3.3 Selection of controls

To each case, up to ten control patients were randomly drawn using the risk set sampling method under the assumption that control patients will be not allowed to have an AMI at the outcome date of their matched cases but they may be eligible to become cases after this date. The study is matched for follow-up time, age (± 5 years), sex and indication ("asthma only"/"COPD only"/"asthma and COPD").

5.3.4 Exposure definition

Current user: A patient is a current user if the outcome day is between the beginning of the most recent prescription and the calculated end date of prescription.

Recent user: A patient is a recent user if the outcome day is during the 91 days following the calculated end date of the most recent prescription.

Past user: A patient is a past user if the outcome day is at least 91 days after the calculated end of prescription.

Current use will be stratified according section 5.2.3.

5.3.5 Confounding

Potential confounders, for which analyses will be adjusted for, are:

- co-morbidities
- Co-medication

An adjusted analysis will be conducted with all potential confounders added to the final model. It can only be applied if as a rule of thumb there are at least 10 events per independent variable in the model. If the number of variables in the model would be too large (< 10 events per variable), selection procedure, including only potential confounders that result in a + or - 5% change of the beta-coefficient of the drug exposure of interest when the individual potential confounder is added to an age/gender adjusted model. If this still results in too many variables, only the potential confounders that change this beta-coefficient most will be included until the maximum number of variables allowed in the model is reached.

5.3.6 Analysis

Conditional logistic regression analysis will be used to estimate the risk of AMI with current use of LABA compared to the control group. The risks will be calculated in terms of odds ratios (OR) with corresponding 95% confidence interval. Adjusted OR for AMI will be estimated by comparing inhaled LABA with the control group (No-LABA) using conditional regression analysis. The calculation will be done with procedure phreg in SAS or the function clogit of the package survival in R.

5.4 Case-crossover study

In the case-crossover study each case acts as its own control.

5.4.1 Study population

All patients of the cohort study, who have developed a myocardial infarction.

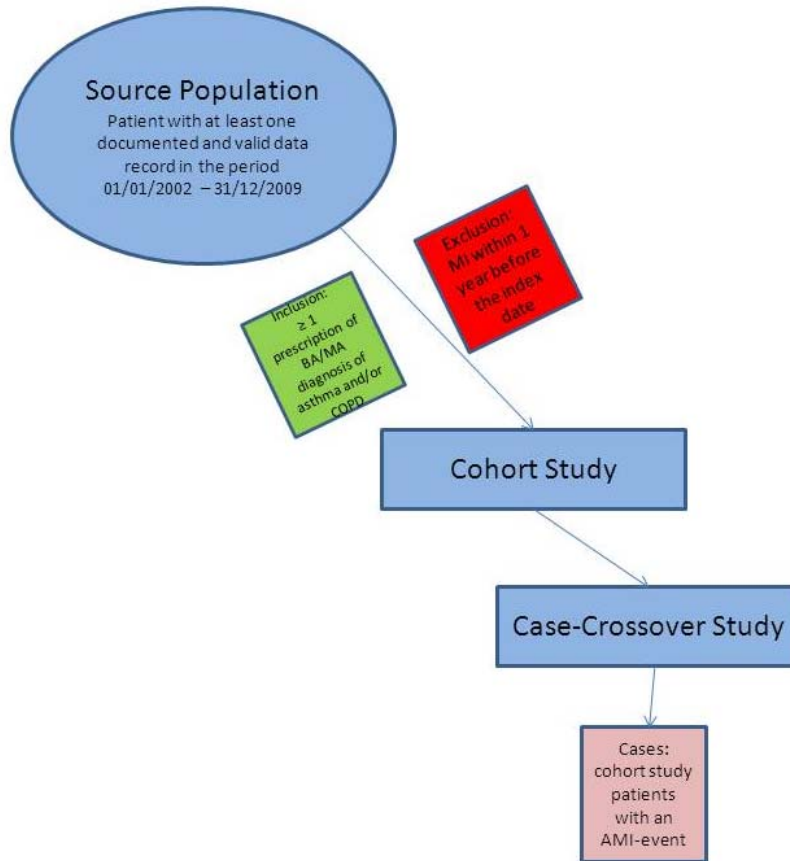


Figure 8: Study Population Case-Control Study

5.4.2 Case window

24 hours for the AMI.

5.4.3 Control window

For each patient the exposure data are compared between the case window and multiple control windows. The control windows are the same weekdays as the case window during the past twelve months.

5.4.4 Analysis

For analysis we use the Nonparametric Multiple Intervals Approach. The model is described in Mittlemann et al. 1995 (58). Conditional logistic regression analysis will be used to estimate the risk of AMI with the use of inhaled LABA and the control group adjusted the various confounding variables. The risks will be calculated in terms of odds ratios (OR) with corresponding 95% confidence interval. The calculation will be done with procedure phreg in SAS or the function clogit of the package survival in R.

6 Sensitivity analyses (optional)

To investigate the influence of several factors important in this association, the following sensitivity analyses are proposed. Within these analyses, the following issues are of high importance (additional stratification of exposure, additional stratification of control groups, oral and i.v. formulations (included as confounders), additional drugs as surrogates for severity). Other issues (additional definition of current, recent, and past use, dynamic assessment of indication, discrimination between fatal and non-fatal AMI, additional co-medication

(included as confounders) and discrimination between incident and non-incident current LABA users are of minor importance only.

6.1 Additional stratification of exposure (high priority)

Since the two inhaled LABA compounds differ with regard to some pharmacokinetic aspects (e.g. onset of bronchodilative effect is much faster for formoterol compared to salmeterol [see introduction]), we will evaluate not only inhaled LABA as whole but also the two compounds separately. As shown by SMART results (10), concomitant usage of ICS in patients receiving salmeterol is crucial and may influence overall mortality significantly. Therefore, we will also assess both inhaled LABA compounds with regard to the concomitant usage of ICS (formoterol with and without ICS/salmeterol with and without ICS). Patients with switches between treatment groups will be counted for each group.

- Inhaled LABA (formoterol or salmeterol) (without ICS but irrespective of any other drugs for COPD/asthma)
- Inhaled LABA (formoterol or salmeterol) and concomitant ICS (fixed combinations)
- Inhaled LABA (formoterol or salmeterol) and concomitant ICS dispensed in two inhalers.

6.2 Additional definition of control groups (high priority)

The following drugs (combinations) are defined as potential control groups for the 3 patient strata.

- Inhaled SAMA only or inhaled SAMA and ICS (no other drugs for COPD/asthma)
- Inhaled SABA only or inhaled SABA and ICS (no other drugs for COPD/asthma)
- Inhaled LAMA only or inhaled LAMA and ICS (no other drugs for COPD/asthma)

6.2.1 Additional Comparisons to be made taking into account additional stratifications of exposure and additional definitions of control group as stated above (high priority):

- Current inhaled LABA (formoterol and salmeterol, without ICS but irrespective of any other drugs for COPD/asthma) vs. current Inhaled LABA (formoterol or salmeterol) and concomitant ICS in a fixed combination
- Current inhaled LABA (without ICS but irrespective of any other drugs for COPD/asthma) vs. current Inhaled LABA (formoterol or salmeterol) and concomitant ICS dispensed in two inhalers
- Current Inhaled LABA (formoterol or salmeterol) and concomitant ICS fixed combinations vs. current Inhaled LABA (formoterol or salmeterol) and concomitant ICS dispensed in two inhalers
- Current Inhaled LABA (formoterol or salmeterol) or current inhaled LABA (formoterol or salmeterol) and concomitant ICS fixed combinations or current inhaled LABA (formoterol or salmeterol) and concomitant ICS dispensed in two inhalers vs. current inhaled SAMA only or inhaled SAMA and ICS (no other drugs for COPD/asthma)
- Current Inhaled LABA (formoterol or salmeterol) or current inhaled LABA (formoterol or salmeterol) and concomitant ICS fixed combinations or current inhaled LABA (formoterol or salmeterol) and concomitant ICS dispensed in two inhalers vs. current inhaled SABA only or inhaled SABA and ICS (no other drugs for COPD/asthma)
- Current Inhaled LABA (formoterol or salmeterol) or current inhaled LABA (formoterol or salmeterol) and concomitant ICS fixed combinations or current inhaled LABA (formoterol or salmeterol) and concomitant ICS dispensed in two inhalers vs. current inhaled LAMA only or inhaled LAMA and ICS (no other drugs for COPD/asthma)
- Current Inhaled formoterol vs current inhaled salmeterol

6.3 Oral and i.v. formulation (high importance)

Formulation with a higher systemic exposure (oral, intravenous application) will be considered as confounders.

6.4 Further definition of current, recent and past use

The duration of the LABA recent and LABA past use will be varied as follows:

Current use	Recent use	Past use
from the beginning of the prescription up to the calculated end date of the prescription	60 days after the calculated end date	More than 60 days after the calculated end date
from the beginning of the prescription up to the calculated end date of the prescription	30 days after the calculated end date	More than 30 days after the calculated end date

The duration of the Non-LABA recent use will be varied as follows:

Current use	Non-Current user
from the beginning of the prescription up to 180 days	180 days after beginning of the prescription

6.5 Fatal vs. non-fatal AMI (low priority)

In the general protocol all AMI cases are taken up in the analysis, as it is challenging to include fatal AMI in all databases due to lack of cause of death. In databases where this is possible, the risk for fatal AMI will be investigated, as well as the risk for non-fatal. (Definition fatal AMI: death within 30 days after the AMI)

6.6 Dynamic assessment of indication (low priority)

Since patients with a diagnosis of asthma may develop COPD within the study period (and vice versa), we have to reproduce this problem in our analysis. Nevertheless, indication for each prescription is documented in some databases only and therefore, a static assessment of indication will be performed in the descriptive studies and in the cohort studies. For defining the indication, longest available period documented in the respective databases (life-time period, if possible) should be considered.

In the sensitivity analysis, a dynamic assessment of indication will be performed every three months for the cohort studies (see figure 4). If a patient has been coded with asthma first and develops COPD afterwards (within the study period), the patient will be grouped before the diagnosis of COPD as an "asthma" patient and starting with the first COPD diagnosis as "asthma and COPD" patient (irrespective whether both diagnoses are coded from that point of time). Same procedure will be performed vice versa (patient with a coded COPD developing asthma). Our approach will result in three patients' strata which might include the same patients in two strata. Therefore, "asthma only" and the "asthma and COPD" cohort as well as the "COPD" and the "asthma and COPD" cohort will not be fully independent. On the other hand, the cohorts of "asthma only" and "COPD only" patients are fully independent.

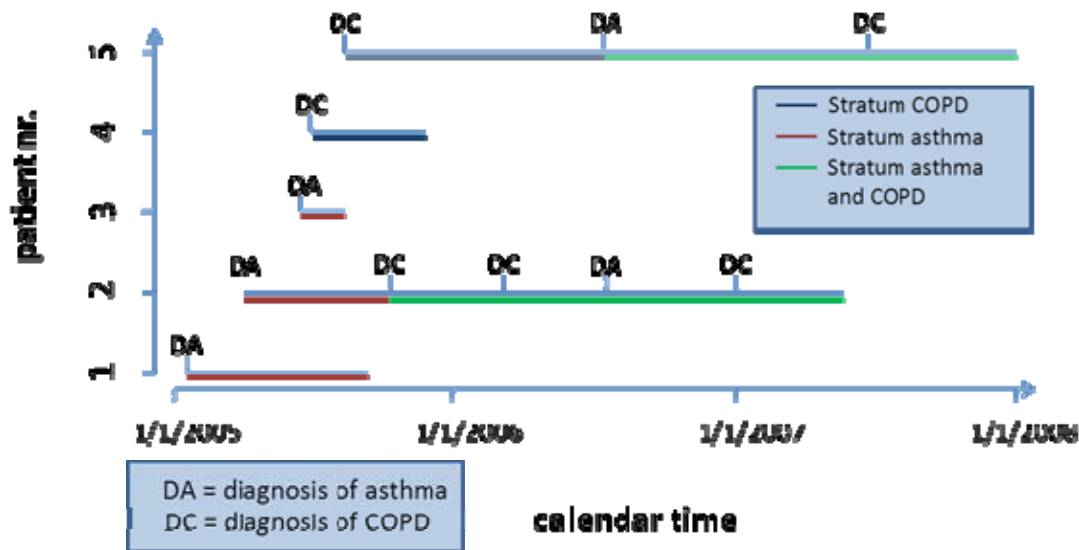


Figure 4: Change of indication: dynamic assessment

Additionally, sequence of diseases will be analysed for the COPD-and-asthma stratum (number of patients diagnosed with asthma followed by a COPD diagnosis and vice versa).

6.7 Additional surrogates for defining disease severity (high priority)

Because the definition of severity with drugs as surrogate parameter is imprecise and treatment guidelines are not useful due to the linkage to the exposure (LABA is the exposure and is an important factor for the definition of the treatment steps), clinical and other endpoints will be used for defining severity for the 3 patient groups as follows (if available in the respective dataset):

6.7.1 For Asthma:

- i.) Controller total asthma medication ratio (61)
- ii.) # of SABA prescriptions/dispensing in prior 12 months / increase in SABA prescriptions in prior 12 month (absolute numbers and/or per cent rise)
- iii.) # of short courses of oral corticosteroids (OCS) or antibiotic prescriptions within two weeks of GP or specialist encounter for respiratory-related diagnosis (ICD class J) as proxy for asthma exacerbation

6.7.2 For COPD:

- i.) # of SABA/SAMA prescriptions/dispensing in prior 12 months / increase in SABA prescriptions in prior 12 month (absolute numbers and/or per cent rise).
- ii.) # of short courses of OCS or antibiotic prescriptions within two week of GP or specialist visit for respiratory-related diagnoses as proxy for moderate COPD exacerbation.

6.7.3 For asthma and COPD patients

i.) # of SABA/SAMA prescriptions/dispensing in prior 12 months / / increase in SABA prescriptions in prior 12 month (absolute numbers and/or per cent rise)

ii.) # of short courses of OCS or antibiotic prescriptions within two week of GP or specialist visit for respiratory-related diagnoses as proxy for moderate COPD / asthma exacerbation.

6.8 Other co-medication

Some co-medication might have only a minor influence on the AMI risk. These factors will also be considered in the sensitivity analysis.

Each parameter should be included as a separate confounder in the analysis.

7 Instrumental Variables

A method that potentially controls for both observed and unobserved confounding is instrumental variable (IV) analysis. An IV is a variable that is strongly related to exposure, and only related to the outcome through exposure. Hence, an IV should neither directly nor indirectly through (unobserved) confounders be associated with the outcome. Throughout the PROTECT project, it will be attempted to develop instrumental variables by WP2; WG2 Confounding. An example of an IV is allocation of treatment in a randomized trial. The allocation is strongly related to actual treatment status, while (due to randomization) it is not related to the outcome, or to (potential) risk factors of the outcome. At this moment progress is made in developing IV analyses methods. However, a detailed research plan to include IV analysis in this protocol is lacking. During the PROTECT project, IV analysis will be performed on the data used for the research described in this protocol.

8 Limitations

The studies described in this protocol are limited by some aspects related to the study designs and the utilized methodologies. First, COPD and asthma were not separated accurately in the coding process (e.g., ICD-miscoding and misdiagnosing). The assignment to COPD or asthma disease groups is based on ICD codes and not on any further free text contained in the records.

Second, in most databases a lethal outcome of MI could not be accurately traced, because there was no linkage between the pharmacoepidemiological databases and the national registers of deaths. In addition, no common definition of lethal MIs exists. Furthermore, little information concerning hospitalizations is available.

Third, our analyses are only based on prescription data. However, inhaled SABA is normally an acute on-demand medication. In praxis, there might result a great period between the prescription and the time of drug intake. Hence, a large uncertainty results concerning the exposure at the time of the MI.

Fourth, the severity of asthma and COPD has to be considered in our analysis as an influence on MI risk cannot be excluded. Unfortunately, severity of these diseases is only rarely depicted in ICD-coding in general and only one database includes lung function parameters (e.g., FEV1). In the most databases in our studies, only the

medication regime can be used as a surrogate parameter for the severity but due to methodological concerns, treatment steps will be used only in the sensitivity analysis.

Fifth, a major limitation is related to data availability and completeness within each data source. Information on important confounders such as socioeconomic status and alcohol and tobacco dependence are not recorded in most databases. In addition, information on OTC drugs (e.g., NSAID) which might be a confounding factor regarding the AMI risk is missing.

Sixth, since patients on a mild asthma (step 1) use only reliever medication on an irregular basis, a 3 months-periods might be not appropriate for these patients.

Seventh, only patients with an AMI within one year before the index date are excluded from the studies. So we have patients with their first-ever AMI and patients with re-infarctions, if the first infarction is more than one year before the index date, in the study population.

Eighth, several coding methods are used for the coding of diseases (ICPC, ICD and readcodes). These methods differ in specificity. ICD coding is generally more specific than ICPC; however the reverse is true in some cases also. There are several ICPC codes which code for several ICD codes. This can also be the case for ICD codes which are not in the confounding tables. This means that there possibly will be adjusted for more diseases in the ICPC codes than for ICD.

Ninth, regarding the definition of patients' strata, we simplify the clinical reality to some extent. We assume, that a patient, who develop a second disease of interest continue to suffer from the first coded disease.

9 Abbreviations

ACQUIP	Ambulatory Care Quality Improvement Project
AE	adverse event
AEMPS	Spanish Medicines Agency
AIS	Additional Information Service
AMI	acute myocardial infarction
ATC	Anatomical Therapeutic Chemical classification system
B2A	beta-2-adrenoceptor agonist
BID	twice a day
BIFAP	Base de datos Informatizada para estudios Framacoepidemiologicos en Atencion Primaria
CEIFE	Spanish Centre for Pharmacoepidemiological Research
CI	confidence interval
COPD	chronic obstructive pulmonary disease
DDD	defined daily dose
FEV1	forced expiratory volume in 1 second
FVC	forced vital capacity
GINA	Global Initiative for Asthma
GOLD	Global Initiative for Chronic Obstructive Lung Disease
GP	General Practitioners
GPRD	General Practice Research Database
HNU	The General Practitioners of Utrecht database
ICPC	international classification of primary care
ICD	international classification of diseases
ICS	inhaled corticosteroids
IDR	incidence density ratio
INPS	In Practice Systems Ltd.
IV	instrumental variable
LABA	long-acting beta-2-adrenoceptor agonist
LAMA	long-acting muscarinic antagonist
LINH	Dutch General Practitioner database
LRJG	Leidsche Rijn Julius Health Centre database
MA	muscarinic antagonist
MPR	medication possession ratio
NHS	National Health Services
PE	pharmacoepidemiological
OR	odds ratio
QD	once a day
RABA	rapid-acting beta-2-adrenoceptor agonist
RCT	randomised controlled trial
Rx	prescription drug
SABA	short-acting beta-2-adrenoceptor agonist
SAE	serious adverse event
SAMA	short-acting muscarinic antagonist
SMART	Salmeterol Multicenter Asthma Research Trial
THIN	The Health Improvement Services
ZGA	The Almere Health Care Database

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11 Annex IV: Amendments

11.1 Amendment 1

Protocol: PROTECT_WP2 Final protocol Beta2_AMI 30 March 2012.doc

Amendment number: N^o 1

Amendments suggested on: 22 August 2012 (see Reasons for amendment)

Amendments finalized on: 13 September 2012

Protocol Owners and Reviewers:

Name	Role
Marietta Rottenkolber ¹	Protocol lead
Sven Schmiedl ³	Protocol backup
Arlene Gallagher ⁴	Protocol reviewer
Xavier Kurz ⁵	Protocol reviewer
Omer de Mol ⁶	Protocol reviewer
Saga Johanson ⁷	Protocol reviewer
Frans Rutten ²	Protocol reviewer
Kourtney Davis ⁸	Protocol reviewer
Ana Sofia Afonso	Protocol reviewer
Marietta Rottenkolber ¹	Database 1 (Bavaria) lead
Joerg Hasford ¹	Database 1 (Bavaria) backup
Miguel Gil ⁹	Database 2 (Bifap) lead
Consuelo Huerta ⁹	Database 2 (Bifap) backup
Ulrik Hesse ¹⁰	Database 3 (DKMA) lead
Frank de Vries ²	Database 3 (DKMA) backup
Paola Primatesta ¹¹	Database 4 (GPRD) lead
Dan Dedman /Jenny Campbell ⁴	Database 4 (GPRD) backup
Eef Voogd ² ,Olaf Klungel ²	Database 5 (Mondriaan) lead
Liset van Dijk ²	Database 5 (Mondriaan) backup
Yolanda Alvarez ⁵	Database 6 (THIN) lead
Ana Ruigomez ¹²	Database 6 (THIN) backup
Mark de Groot ² and Raymond Schlienger ¹¹	WG1 colead
Olaf Klungel ² and Robert Reynolds ¹³	WP2 coleads

1 Institute for Medical Information Sciences, Biometry and Epidemiology, Ludwig-Maximilians-Universitaet Munich, Germany (LMU Muenchen)

2 Universiteit Utrecht, Utrecht, The Netherlands (UU)

3 Philipp Klee-Institute for Clinical Pharmacology, HELIOS Clinic Wuppertal, Wuppertal, Germany / Department of Clinical Pharmacology, School of Medicine, Faculty of Health, Witten/Herdecke University, Germany

4 General Practice Research Database, London, United Kingdom (GPRD)

5 European Medicines Agency, London, United Kingdom (EMA)

6 Genzyme Europe B.V., The Netherlands

7 AstraZeneca AB, Sweden (AZ)

8 GlaxoSmithKline Research and Development LTD, Belgium (GSK)

9 Agencia Espanola de Medicamentos y Productos Sanitarios, Madrid, Spain (AEMPS)

10 Lægemedelstyrelsen (Danish Medicines Agency), Copenhagen, Denmark (DKMA)

11 Novartis Pharma AG, Basel, Switzerland (Novartis)

12 Fundación Centro Español de Investigación Farmacoepidemiológica, Madrid, Spain (CEIFE)

Reason(s) for amendment:

This protocol amendment serves to the following purposes:

- a) Change of indication assessment: In several databases, there is no clear linkage between indication and prescription. For homogeneity of procedures, static assessment (as done in the descriptive study) should be performed in the cohort study by all databases. In sensitivity analysis, impact of using dynamic assessment of indications can be studied.
- b) Fatal MI will be defined as “death within 30 days after AMI” as a surrogate
- c) The issue, that treatment steps as surrogate for disease severity are too closely related to the LABA exposure for a meaningful analysis has been discussed already for several times. Nevertheless, all participants agreed to move treatment steps to the sensitivity analysis, i.e. severity of disease is not any longer included as confounder in the main analysis. Obviously, drugs used for asthma treatment will be considered as confounders.
- d) Clarification of definitions (e.g., comparison groups)

Protocol Section(s) suggested for amendment

11.1.1 a) Indication assessment

5.1.3.2 Change from:

In the second step, measurements considered in 1.), 3.), 4.), 5.) of the first step will be separately performed for the three patients’ strata: i.) patients with asthma only, ii.) patients with COPD only and iii.) patients with COPD and asthma (if available in the database). Patients suffering neither from asthma nor from COPD will be excluded from further analysis (due to heterogeneity of diseases). (Remark: in 2.) of the first step of the descriptive study, the definition of the strata already reflects the indications. Therefore, stratification by indication for this issue is redundant.)

The denominator for the second step of the descriptive analysis is the number of patients in the respective stratum.

5.1.3.2 Change to:

In the second step, measurements considered in 1.), 3.), 4.), 5.) of the first step will be separately performed for the three patients’ strata: i.) patients with asthma only, ii.) patients with COPD only and iii.) patients with COPD and asthma (if available in the database). **Since patients with a diagnosis of asthma may develop COPD within the study period (and vice versa), we have to reproduce this problem in our analysis. Nevertheless, indication for each prescription is documented in some databases only and therefore, a static assessment of indication will be performed. Static assessment means that the indication is defined at the date of last prescription within the study period assessing the whole study period.**

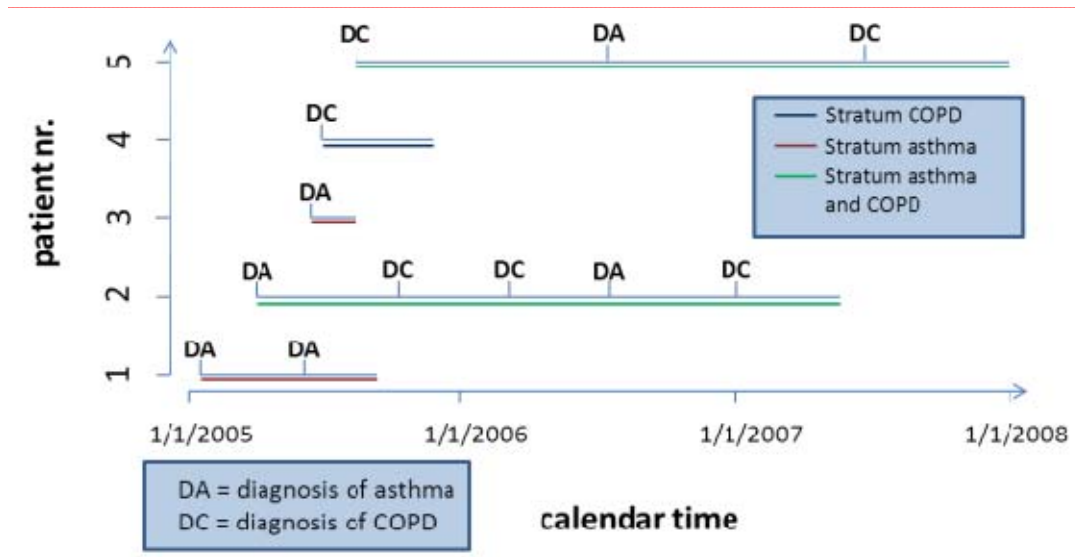


Figure 2: Change of indication: static assessment

Patients suffering neither from asthma nor from COPD will be excluded from further analysis (due to heterogeneity of diseases). (Remark: in 2.) of the first step of the descriptive study, the definition of the strata already reflects the indications. Therefore, stratification by indication for this issue is redundant.)

The denominator for the second step of the descriptive analysis is the number of patients in the respective stratum.

5.2.5 Change from

Since patients with a diagnosis of asthma may develop COPD within the study period (and vice versa), we have to reproduce this problem in our analysis. A dynamic assessment of indication will be performed every three months for the cohort studies (see figure 4). If a patient has been coded with asthma first and develops COPD afterwards (within the study period), the patient will be grouped before the diagnosis of COPD as an "asthma" patient and starting with the first COPD diagnosis as "asthma and COPD" patient (irrespective whether both diagnoses are coded from that point of time). Same procedure will be performed vice versa (patient with a coded COPD developing asthma). Our approach will result in three patients' strata which might include the same patients in two strata. Therefore, "asthma only" and the "asthma and COPD" cohort as well as the "COPD" and the "asthma and COPD" cohort will not be fully independent. On the other hand, the cohorts of "asthma only" and "COPD only" patients are fully independent.

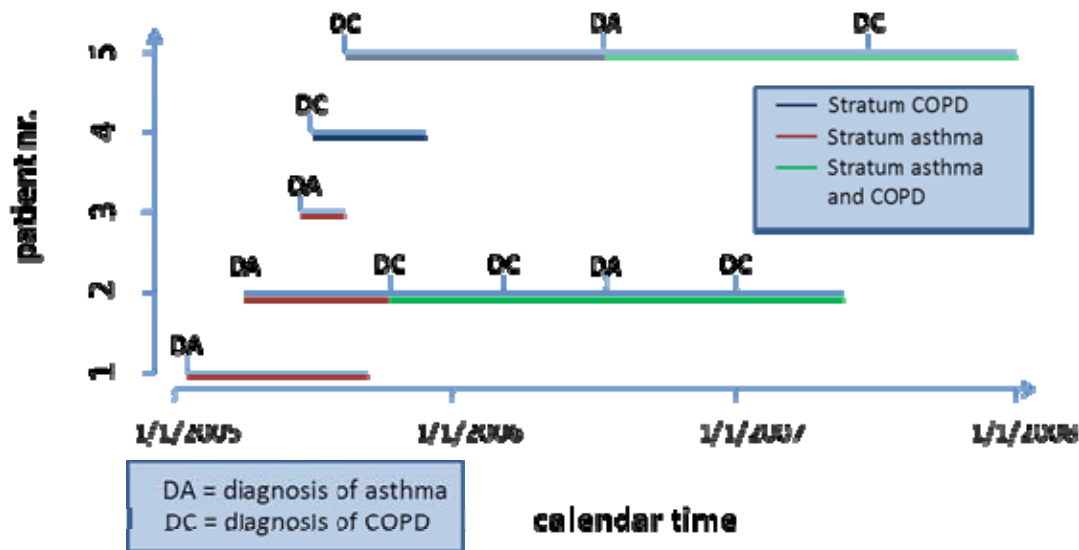


Figure 3: Change of indication: dynamic assessment

5.2.5 Change to:

Since patients with a diagnosis of asthma may develop COPD within the study period (and vice versa), we have to reproduce this problem in our analysis. Nevertheless, indication for each prescription is documented in some databases only and therefore, a static assessment of indication will be performed as described in section 5.1.3.2 Within the sensitivity analysis, a dynamic assessment of indication will be performed.

Sensitivity analysis 6.6: Since patients with a diagnosis of asthma may develop COPD within the study period (and vice versa), we have to reproduce this problem in our analysis. Nevertheless, indication for each prescription is documented in some databases only and therefore, a static assessment of indication will be performed in the descriptive studies and in the cohort studies.

In the sensitivity analysis, a dynamic assessment of indication will be performed every three months for the cohort studies (see figure 4). If a patient has been coded with asthma first and develops COPD afterwards (within the study period), the patient will be grouped before the diagnosis of COPD as an “asthma” patient and starting with the first COPD diagnosis as “asthma and COPD” patient (irrespective whether both diagnoses are coded from that point of time). Same procedure will be performed vice versa (patient with a coded COPD developing asthma). Our approach will result in three patients’ strata which might include the same patients in two strata. Therefore, “asthma only” and the “asthma and COPD” cohort as well as the “COPD” and the “asthma and COPD” cohort will not be fully independent. On the other hand, the cohorts of “asthma only” and “COPD only” patients are fully independent.

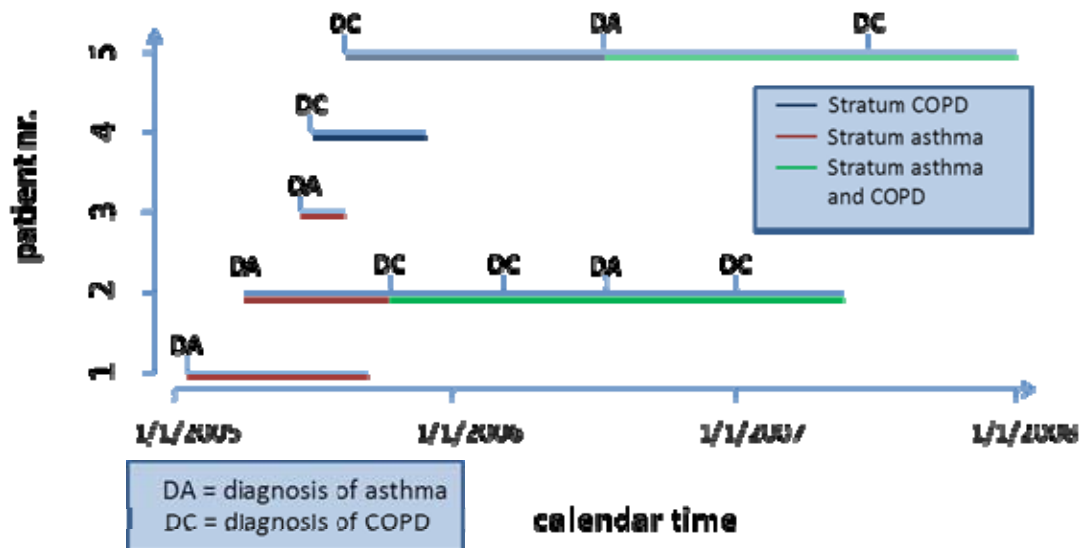


Figure 4: Change of indication: dynamic assessment

11.1.2 b) Fatal MI

Change from:

In the general protocol all AMI cases are taken up in the analysis, as it is challenging to include fatal AMI in all databases due to lack of cause of death. In databases where this is possible, the risk for fatal AMI will be investigated, as well as the risk for non-fatal.

Change to:

In the general protocol all AMI cases are taken up in the analysis, as it is challenging to include fatal AMI in all databases due to lack of cause of death. In databases where this is possible, the risk for fatal AMI will be investigated, as well as the risk for non-fatal. (Definition fatal AMI: death within 30 days after the AMI)

11.1.3 c) Severity

5.2.6.2 Change from:

Since influence on MI risk cannot be excluded, the severity of asthma and COPD has to be included in our analysis as a confounding factor (59). Unfortunately, severity of both diseases is only rarely depicted in ICD-coding in general. Furthermore, clinically relevant parameters describing disease severity (e.g. lung function parameters, number of hospitalisations) are not documented in most databases. Therefore, we decided to use guideline's treatment steps focussing on drugs as surrogate for severity as already done by others (8).

Regarding the numerous drugs used in COPD and asthma and possible drug combinations, treatment steps can only be judged as a very rough surrogate for disease severity. Additionally, drugs not primarily recommended in the guidelines (and depicted in the treatment steps) will also be used (e.g. theophylline in COPD patients). Therefore we included the group "combinations not listed" in our treatment steps. Furthermore, prescription behaviour might have changed within the last years affecting the study population (e.g. LABA usage without ICS has been re-assessed after the SMART trial (10)). Therefore, we did change the current asthma treatment step 3 consisting of a fixed combination of LABA/ICS in "LABA or LABA/low dose ICS". In summary, there are several severe limitations regarding the usage of treatment steps for assessing disease severity.

Furthermore, focussing on drugs only and non-considering of clinical issues can only give a limited impression of severity as stated also in the GINA guidelines: “It is important to recognize, however, that asthma severity involves both the severity of the underlying disease and its responsiveness to treatment. Thus, asthma could present with severe symptoms and airflow obstruction, but become completely controlled with low-dose treatment”. Regarding the relevance of clinical endpoints we want to use clinical parameters (if available) to refine disease severity assessment in the sensitivity analysis for asthma and COPD patients separately. For the other patients included in our study (COPD & asthma) relevant clinical issues (e.g. hospitalisation) will also be defined.

Since severity will change over time, we will (re)assess severity regarding 3-months intervals. Additionally, treatment steps according to the guidelines can be used only in patients with a diagnosis of asthma or COPD. For patients suffering from both diseases (our 3rd cohort) simplified treatment steps will be used for defining severity.

Definition of treatment steps for asthma:

According to recent guidelines published by the Global Initiative for Asthma ([GINA] see figure 4 (1)), drugs can be used for defining treatment steps. In general, asthma (and COPD) drugs can be divided in reliever compounds used as needed medication due to a rapid bronchodilation and in controller medication which is used on a regular base (several long-acting, slow-acting mechanisms, e.g. anti-inflammation). In treatment step 1, reliever medication only is given to the patient but in treatment step 2, controller medication is started (and reliever medication is added on an “as needed base” by the patient).

		Step 1	Step 2	Step 3	Step 4	Step 5
Asthma education Environmental control						
		As needed rapid-acting β_2 -agonist	As needed rapid-acting β_2 -agonist			
Controller options			Select one	Select one	Add one or more	Add one or both
			Low-dose inhaled ICS [†]	Low-dose ICS plus long-acting β_2 -agonist	Medium-or high-dose ICS plus long-acting β_2 -agonist	Oral glucocorticosteroid (lowest dose)
			Leukotriene modifier [‡]	Medium-or high-dose ICS	Leukotriene modifier	Anti-IgE treatment
				Low-dose ICS plus leukotriene modifier	Sustained release theophylline	
				Low-dose ICS plus sustained release theophylline		

[†] Inhaled glucocorticosteroids. [‡] Receptor antagonist or synthesis inhibitors.

Figure 4: Asthma treatment according to the GINA guidelines (preferred controller options are shown in shadowed boxes; figure taken from Hoshino et al. (60) Please note: Whereas “rapid-acting” B2A are stated as “as-needed” medication the GINA guidelines (1), “short-acting” B2A are stated as “as-needed” medication in the British Guideline (4) (see figure 5).

Whereas in some guidelines (e.g. GINA (1)) RABA (rapid acting B2A) are stated as reliever medication in other guidelines (e.g. British guideline on the Management of Asthma (4), see figure 5) SABA are recommended.

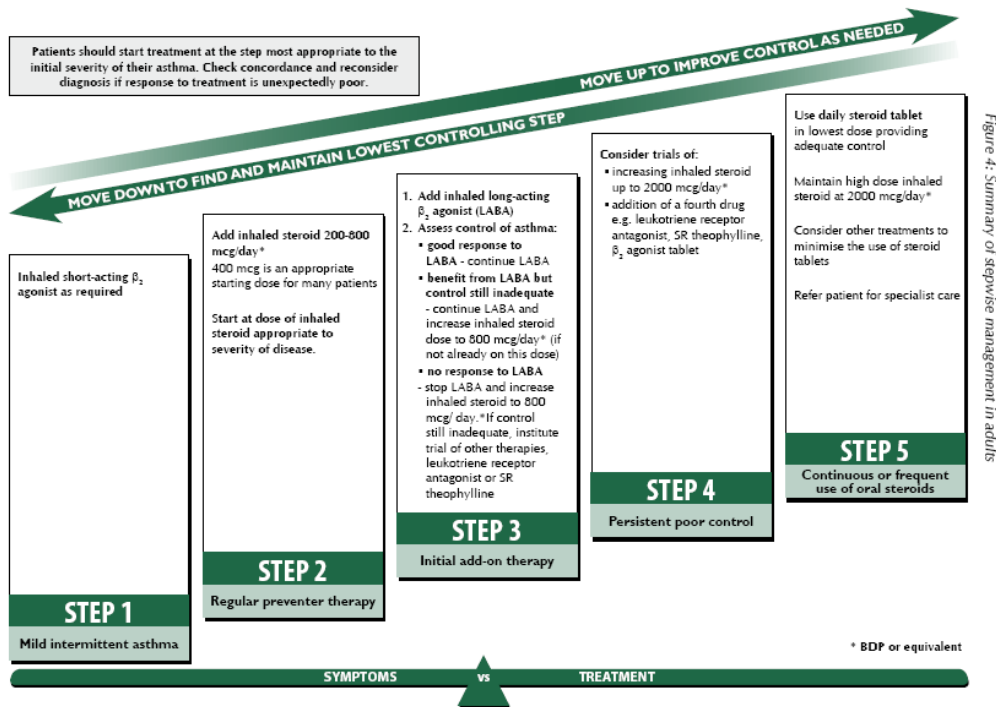


Figure 5: Asthma treatment according to the British Guideline on the Management of Asthma (May 2008, revised May 2011(4)). Please note: Whereas “short-acting” B2A are stated as “as-needed” medication in the British Guideline, “rapid-acting” B2A are stated as “as-needed” medication the GINA guidelines (1) (see figure 4).

As discussed above, formoterol is a rapid-acting LABA (and a member of the RABA class) and might be used in treatment step 1 as reliever medication (see e.g. GINA (1)). On the other hand, formoterol should be used - as already discussed for LABAs and according to the guidelines – in combination with ICS only. Focussing on the effect duration (and the combined usage of LABA and ICS which is widely stated as treatment step 3), we decided to use the conventional classification system (SABA and LABA) as stated e.g. in the recently published British guideline on the Management of Asthma (4) (see figure 5). Thus, we want to reduce confusion regarding formoterol and all patients using this compound will be included in our analysis as at least treatment step 3.

Treatment step 1*: Reliever medication only: SABA, SAMA, short-acting theophylline

Treatment step 2: Low dose ICS or leukotriene modifier (plus reliever medication)

Treatment step 3: LABA or LABA/low dose ICS or medium /high-dose ICS or low-dose ICS plus leukotriene modifier or low-dose ICS plus sustained release theophylline (plus reliever medication)

Treatment step 4: Treatment step 3 plus one or more of the following: LABA and medium- or high-dose ICS and/or leukotriene modifier and/or sustained release theophylline

Treatment step 5**: Addition of oral glucocorticosteroid to Step 4

Treatment step 6**: Addition of omalizumab to step 5

Treatment step 7: combinations not listed above

*Since inhaled SABA is the preferred drug class within the reliever medication group and other reliever drug (classes) e.g. oral SABA and theophylline are typically used in patients with a more severe asthma due to the increased systemic drug exposure, treatment step 1 will be split in two groups (inhaled, oral reliever medication) which will also be considered for the other treatment steps in the sensitivity analysis.

**Since omalizumab should only be used as a “last” option, step 5 according to GINA has been divided in two steps (chronic oral corticosteroids [duration of prescription at least 30 days] and omalizumab).

As stated above, time course of severity changes has to be taken into account. Since seasonal influences are well-known particularly for extrinsic (allergic) asthma, a higher asthma severity during spring and summer may occur but in autumn / winter, asthma medication might be reduced. Due to these changes, severity assessment of asthma patients will be performed on a 3-months interval (quarter).

Definition of treatment steps for COPD

Using a current guideline of the Global initiative for chronic Obstructive Lung Disease (2) (see figure 6), drug therapy can also be used for a rough estimation of the severity of COPD. As in asthma patients treatment of COPD is started using reliever medication “as needed” and by reaching treatment step 2, maintenance medication is given on a regular base (and reliever medication as needed).

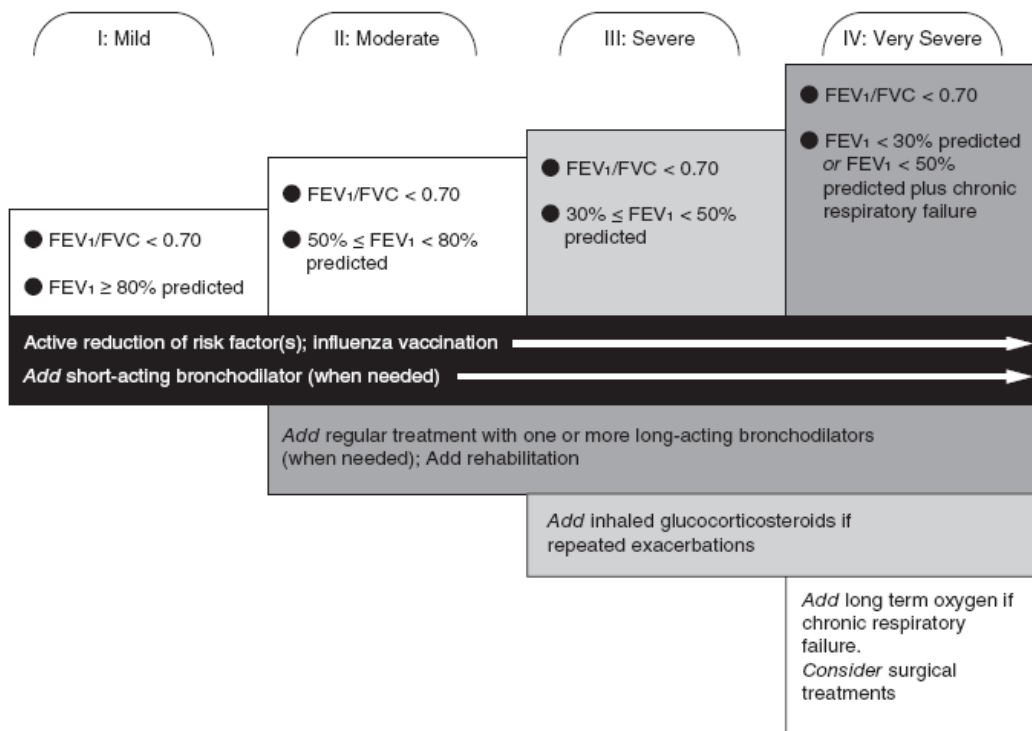


Figure 6: COPD treatment according to GOLD (figure taken from Hoshino et al. (60))

Treatment step 1*: Reliever medication only: SABA, SAMA, short-acting theophylline

Treatment step 2: LAMA or LABA (plus reliever medication)

Treatment step 3: Treatment step 2 plus inhaled corticosteroids

Treatment step 4: Addition of systemic corticosteroids (usually just for acute exacerbations)

Treatment step 5: combinations not listed above (e.g. slow-release theophylline)

*Whereas inhaled reliever medication is stated as preferred treatment, other reliever drug (classes) e.g. oral SAMA, SABA and theophylline are typically used in patients with a more severe COPD. Due to the increased systemic drug exposure, treatment step 1 will be split in two groups (inhaled, oral reliever medication) which will also be considered for the other treatment steps in the sensitivity analysis.

Since non-drug related issues are documented in some databases only, we will use oxygen therapy and surgical interventions defining very severe COPD in the sensitivity analysis only.

As for asthma, time change of severity steps has to be taken into account. From a pathophysiological perspective, COPD is a chronic and progressive disease in terms of a subtle decrease of lung function parameters and seasonal aspects in terms of allergic compounds might not be as relevant as in asthmatic patients. On the other hand, exacerbations may also cause an only limited worsening of lung function parameters with a clinical improvement after some days (and a reduction in drug therapy). Therefore, severity assessment will be performed on a regular base as for asthma and a 3 months period seems to appropriate.

Definition of treatment steps for patients with COPD and Asthma

Regarding both guidelines (GINA (1) and GOLD (2)), relevant differences have to be mentioned. In asthma patients, ICS is given as first controller medication and LABA usage without ICS is not recommended (at least after the SMART trial (10)) due to the inflammatory etiology of asthma. In contrast, LABA usage without ICS is recommended in COPD patients and ICS is given only to patients suffering from severe COPD. Therefore the following simplified treatment steps can only be used as are very rough assessment of disease severity in patients suffering from these two diseases.

Treatment step 1*: reliever medication only (compounds see above)

Treatment step 2: reliever medication plus addition of long-acting bronchodilative agents (LAMA and /or LABA) and/or inhaled corticosteroids

Treatment step 3: addition of systemic corticosteroids

*Whereas inhaled reliever medication is stated as preferred treatment, other reliever drug (classes) e.g. oral SAMA, SABA and theophylline or nebulized reliever treatments are typically used in patients with a more severe COPD/asthma who have issues with inhaler technique perhaps due to comorbidities and functional status. Due to the increased systemic drug exposure, treatment step 1 will be split in two groups (inhaled, oral reliever medication) which will also be considered for the other treatment steps in the sensitivity analysis.

As for asthma and COPD, severity assessment will be performed on a 3 months period.

5.2.6.2 Change to:

Since influence on MI risk cannot be excluded, the severity of asthma and COPD has to be included in our analysis as a confounding factor (59). Unfortunately, severity of both diseases is only rarely depicted in ICD-coding in general. Furthermore, clinically relevant parameters describing disease severity (e.g. lung function parameters, number of hospitalisations) are not documented in most databases. **Treatment steps could be useful for defining disease severity but since LABA is used in most treatment steps (particular in asthma patients), methodological aspects limit the results to a he extent. Therefore, treatment steps will be used in the sensitivity analysis only.**

6.7 Sensitivity analysis Since an influence on MI risk cannot be excluded, the severity of asthma and COPD has to be included in our analysis as a confounding factor (59). Unfortunately, severity of both diseases is only rarely depicted in ICD-coding in general. Furthermore, clinically relevant parameters describing disease severity (e.g. lung function parameters, number of hospitalisations) are not documented in most databases. Treatment steps could be useful for defining disease severity but since LABA is used in most treatment steps (particular in asthma patients), methodological aspects limit the results to a huge extent. Therefore, treatment steps will be used in the sensitivity analysis only (8).

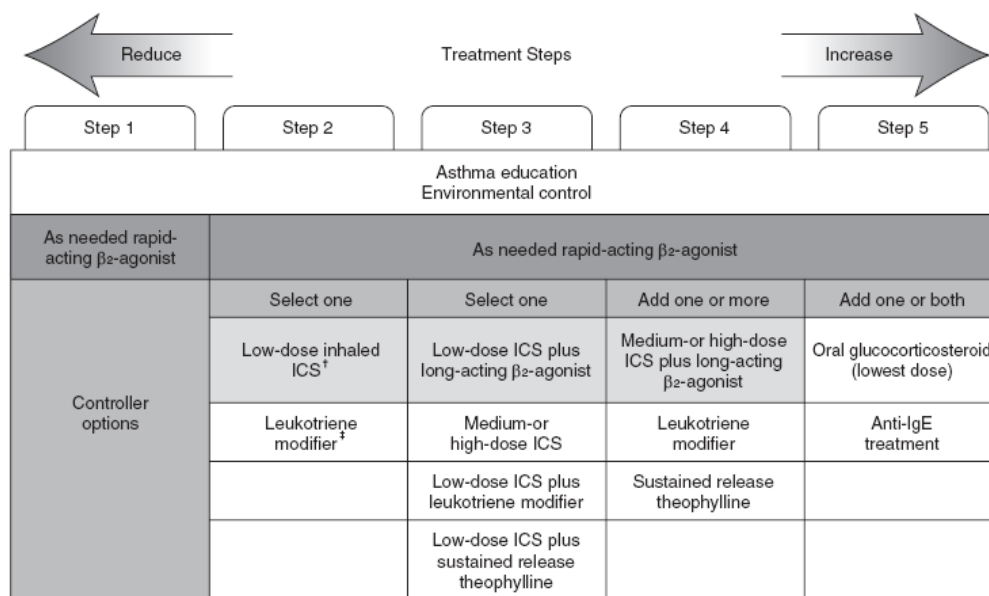
Regarding the numerous drugs used in COPD and asthma and possible drug combinations, treatment steps can only be judged as a very rough surrogate for disease severity. Additionally, drugs not primarily recommended in the guidelines (and depicted in the treatment steps) will also be used (e.g. theophylline in COPD patients). Therefore we included the group “combinations not listed” in our treatment steps. Furthermore, prescription behaviour might have changed within the last years affecting the study population (e.g. LABA usage without ICS has been re-assessed after the SMART trial (10)). Therefore, we did change the current asthma treatment step 3 consisting of a fixed combination of LABA/ICS in “LABA or LABA/low dose ICS”. In summary, there are several severe limitations regarding the usage of treatment steps for assessing disease severity.

Furthermore, focussing on drugs only and non-considering of clinical issues can only give a limited impression of severity as stated also in the GINA guidelines: “It is important to recognize, however, that asthma severity involves both the severity of the underlying disease and its responsiveness to treatment. Thus, asthma could present with severe symptoms and airflow obstruction, but become completely controlled with low-dose treatment”. Regarding the relevance of clinical endpoints we want to use clinical parameters (if available) to refine disease severity assessment in the sensitivity analysis for asthma and COPD patients separately. For the other patients included in our study (COPD & asthma) relevant clinical issues (e.g. hospitalisation) will also be defined.

Since severity will change over time, we will (re)assess severity regarding 3-months intervals. Additionally, treatment steps according to the guidelines can be used only in patients with a diagnosis of asthma or COPD. For patients suffering from both diseases (our 3rd cohort) simplified treatment steps will be used for defining severity.

Definition of treatment steps for asthma:

According to recent guidelines published by the Global Initiative for Asthma ([GINA] see figure 4 (1)), drugs can be used for defining treatment steps. In general, asthma (and COPD) drugs can be divided in reliever compounds used as needed medication due to a rapid bronchodilation and in controller medication which is used on a regular base (several long-acting, slow-acting mechanisms, e.g. anti-inflammation). In treatment step 1, reliever medication only is given to the patient but in treatment step 2, controller medication is started (and reliever medication is added on an “as needed base” by the patient).



[†] Inhaled glucocorticosteroids. [‡] Receptor antagonist or synthesis inhibitors.

Figure 4: Asthma treatment according to the GINA guidelines (preferred controller options are shown in shadowed boxes; figure taken from Hoshino et al. (60) Please note: Whereas “rapid-acting” B2A are stated as “as-needed” medication the GINA guidelines (1), “short-acting” B2A are stated as “as-needed” medication in the British Guideline (4) (see figure 5).

Whereas in some guidelines (e.g. GINA (1)) RABA (rapid acting B2A) are stated as reliever medication in other guidelines (e.g. British guideline on the Management of Asthma (4), see figure 5) SABA are recommended.

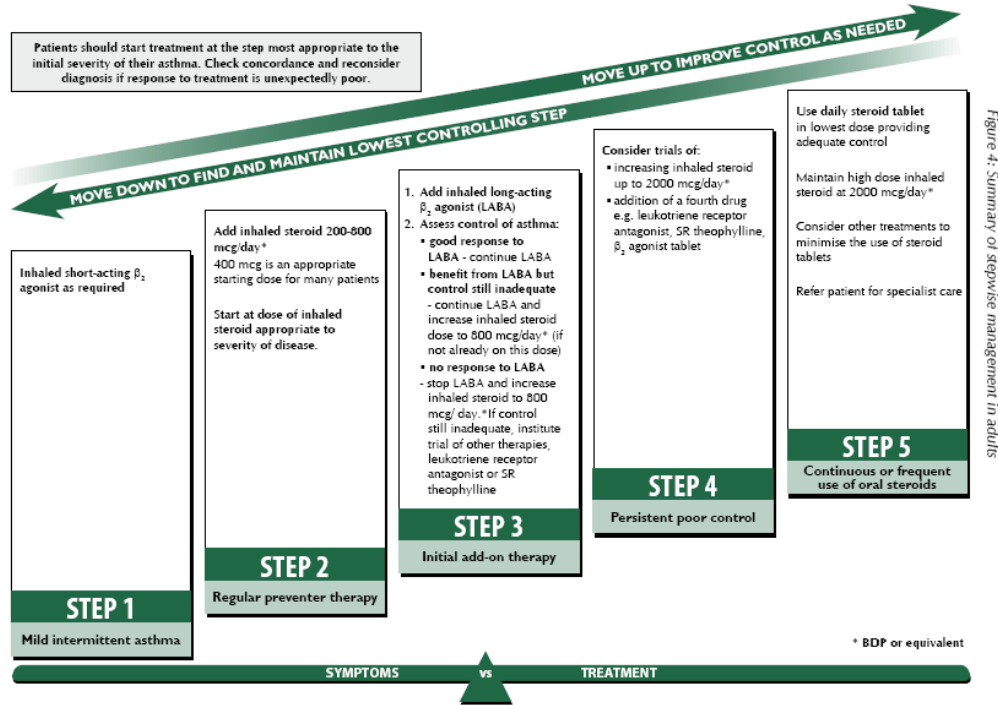


Figure 5: Asthma treatment according to the British Guideline on the Management of Asthma (May 2008, revised May 2011(4)). Please note: Whereas “short-acting” B2A are stated as “as-needed” medication in the British Guideline, “rapid-acting” B2A are stated as “as-needed” medication the GINA guidelines (1) (see figure 4).

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Treatment step 1*: Reliever medication only: SABA, SAMA, short-acting theophylline

Treatment step 2: Low dose ICS or leukotriene modifier (plus reliever medication)

Treatment step 3: LABA or LABA/low dose ICS or medium /high-dose ICS or low-dose ICS plus leukotriene modifier or low-dose ICS plus sustained release theophylline (plus reliever medication)

Treatment step 4: Treatment step 3 plus one or more of the following: LABA and medium- or high-dose ICS and/or leukotriene modifier and/or sustained release theophylline

Treatment step 5**: Addition of oral glucocorticosteroid to Step 4

Treatment step 6**: Addition of omalizumab to step 5

Treatment step 7: combinations not listed above

*Since inhaled SABA is the preferred drug class within the reliever medication group and other reliever drug (classes) e.g. oral SABA and theophylline are typically used in patients with a more severe asthma due to the increased systemic drug exposure, treatment step 1 will be split in two groups (inhaled, oral reliever medication) which will also be considered for the other treatment steps in the sensitivity analysis.

**Since omalizumab should only be used as a “last” option, step 5 according to GINA has been divided in two steps (chronic oral corticosteroids [duration of prescription at least 30 days] and omalizumab).

As stated above, time course of severity changes has to be taken into account. Since seasonal influences are well-known particularly for extrinsic (allergic) asthma, a higher asthma severity during spring and summer may occur but in autumn / winter, asthma medication might be reduced. Due to these changes, severity assessment of asthma patients will be performed on a 3-months interval (quarter).

Definition of treatment steps for COPD

Using a current guideline of the Global initiative for chronic Obstructive Lung Disease (2) (see figure 6), drug therapy can also be used for a rough estimation of the severity of COPD. As in asthma patients treatment of COPD is started using reliever medication “as needed” and by reaching treatment step 2, maintenance medication is given on a regular base (and reliever medication as needed).

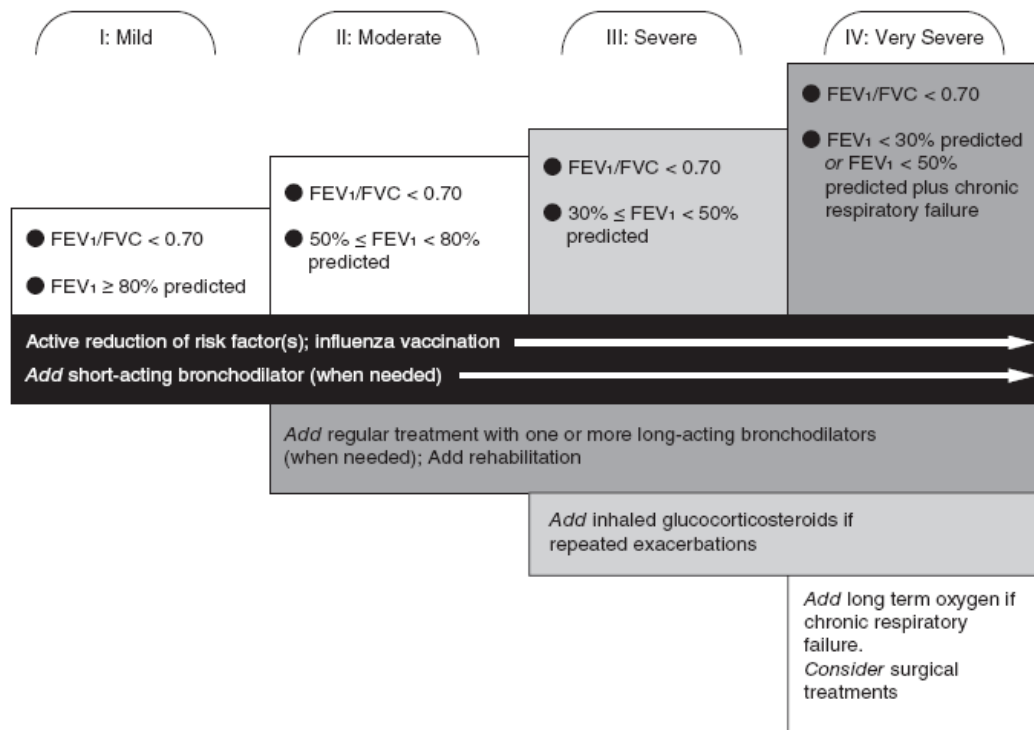


Figure 6: COPD treatment according to GOLD (figure taken from Hoshino et al. (60))

Treatment step 1*: Reliever medication only: SABA, SAMA, short-acting theophylline

Treatment step 2: LAMA or LABA (plus reliever medication)

Treatment step 3: Treatment step 2 plus inhaled corticosteroids

Treatment step 4: Addition of systemic corticosteroids (usually just for acute exacerbations)

Treatment step 5: combinations not listed above (e.g. slow-release theophylline)

*Whereas inhaled reliever medication is stated as preferred treatment, other reliever drug (classes) e.g. oral SAMA, SABA and theophylline are typically used in patients with a more severe COPD. Due to the increased systemic drug exposure, treatment step 1 will be split in two groups (inhaled, oral reliever medication) which will also be considered for the other treatment steps in the sensitivity analysis.

Since non-drug related issues are documented in some databases only, we will use oxygen therapy and surgical interventions defining very severe COPD in the sensitivity analysis only.

As for asthma, time change of severity steps has to be taken into account. From a pathophysiological perspective, COPD is a chronic and progressive disease in terms of a subtle decrease of lung function parameters and seasonal aspects in terms of allergic compounds might not be as relevant as in asthmatic patients. On the other hand, exacerbations may also cause an only limited worsening of lung function parameters with a clinical improvement after some days (and a reduction in drug therapy). Therefore, severity assessment will be performed on a regular base as for asthma and a 3 months period seems to appropriate.

Definition of treatment steps for patients with COPD and Asthma

Regarding both guidelines (GINA (1) and GOLD (2)), relevant differences have to be mentioned. In asthma patients, ICS is given as first controller medication and LABA usage without ICS is not recommended (at least after the SMART trial (10)) due to the inflammatory etiology of asthma. In contrast, LABA usage without ICS is recommended in COPD patients and ICS is given only to patients suffering from severe COPD. Therefore the following simplified treatment steps can only be used as a very rough assessment of disease severity in patients suffering from these two diseases.

Treatment step 1*: reliever medication only (compounds see above)

Treatment step 2: reliever medication plus addition of long-acting bronchodilative agents (LAMA and /or LABA) and/or inhaled corticosteroids

Treatment step 3: addition of systemic corticosteroids

*Whereas inhaled reliever medication is stated as preferred treatment, other reliever drug (classes) e.g. oral SAMA, SABA and theophylline or nebulized reliever treatments are typically used in patients with a more severe COPD/asthma who have issues with inhaler technique perhaps due to comorbidities and functional status. Due to the increased systemic drug exposure, treatment step 1 will be split in two groups (inhaled, oral reliever medication) which will also be considered for the other treatment steps in the sensitivity analysis.

As for asthma and COPD, severity assessment will be performed on a 3 months period.

11.1.4 d) Other changes

5.1 Change from:

Information on the use of inhaled LABA, the indication (without BIFAP), the patient characteristics, and the frequency of the outcome (first AMI within the study period) will be evaluated in each individual database. The descriptive study will be performed in two steps. In the first step, all measurements will be calculated for the whole study population. In the second step, all measurements will be separately calculated for the three patients' strata: patients with asthma only, patients with COPD only and patients with asthma and COPD.

5.1 Change to:

Information on the use of inhaled LABA, the indication (without BIFAP), the patient characteristics, the frequency of the outcome (first AMI within the study period), **and relevant co-morbidities and co-medication (optional, used as confounders in the cohort study)** will be evaluated in each individual database. The descriptive study will be performed in two steps. In the first step, all measurements will be calculated for the whole study population. In the second step, all measurements will be separately calculated for the three patients' strata: patients with asthma only, patients with COPD only and patients with asthma and COPD.

5.1.2 Change from:

In our analysis we will focus on the two inhaled LABA compounds, formoterol and salmeterol, without restriction of concomitant medication. Combined drugs consisting of formoterol and salmeterol will also be considered as exposure. Bambuterol, an oral LABA, will not be considered in our analysis due to a much smaller number of prescriptions compared to inhaled LABA and a higher systemic exposure influencing the risk for occurrence of cardiac side effects. Patients with switches between treatment groups will be counted for each group.

- Exposure: Inhaled LABA (Formoterol, Salmeterol [including combination drugs]) irrespective of other treatments
- Control group: No LABA but at least one of the following
 - o Inhaled LAMA
 - o Inhaled SAMA
 - o Inhaled SABA

The control group will be built by all patients receiving at least one Rx of LAMA, SAMA or SABA as a whole. A more detailed analysis (stratification of control drugs) will be performed in the sensitivity analysis.

5.1.2 Change to

In our analysis we will focus on the two inhaled LABA compounds, formoterol and salmeterol, without restriction of concomitant medication. Combined drugs consisting of formoterol **or** salmeterol will also be considered as exposure. Bambuterol, an oral LABA, will not be considered **as exposure** in our analysis due to **an expected** much smaller number of prescriptions compared to inhaled LABA and a higher systemic exposure influencing the risk for occurrence of cardiac side effects. Patients with switches between treatment groups will be counted for each group.

- **Exposure:** Inhaled LABA (Formoterol, Salmeterol [including combination drugs]) **irrespective of other treatments**
- Control group: No LABA but at least one of the following
 - o Inhaled LAMA
 - o Inhaled SAMA
 - o Inhaled SABA

The control group will be built by all patients receiving at least one Rx of LAMA, SAMA or SABA as a whole. A more detailed analysis (stratification of control drugs) will be performed in the sensitivity analysis.

5.2.1 Change from:

All patients, who received at least one prescription of an inhaled LABA, SABA, LAMA, SAMA (see appendix 1) and with coded diagnosis of asthma and/or COPD during the study period, will be included in the study. Cohort entry is the first new prescription of an inhaled B2A or an inhaled MA for the patient between January 1st 2002 and December 31st 2009, after one year of valid data with no documented incidence of AMI (this event free year can also be before January 1st 2002, when data is available).

5.2.1 Change to:

All patients, who received at least one prescription of an inhaled LABA and/or inhaled SABA and/or inhaled LAMA and/or inhaled SAMA (see appendix 1) and with coded diagnosis of asthma and/or COPD during the study period, will be included in the study. Cohort entry is the first new prescription of an inhaled B2A or an inhaled MA for the patient between January 1st 2002 and December 31st 2009, after one year of valid data with no documented incidence of AMI (this event free year can also be before January 1st 2002, when data is available).

5.2.6.1 Change from:

- Age and sex
- Asthma/COPD disease severity (drugs defined by treatment steps [see above and 7.6.1])
- Other factors influencing AMI risk (see 7.5 and 7.6.2-7.6.5)

An adjusted analysis will be conducted with all potential confounders added to the final model. It can only be applied if as a rule of thumb there are at least 10 events per independent variable in the model. If the number of variables in the model would be too large (< 10 events per variable), selection procedure, including only potential confounders that result in a + or - 5% change of the beta-coefficient of the drug exposure of interest when the individual potential confounder is added to an age/gender adjusted model. If this still results in too many variables, only the potential confounders that change this beta-coefficient most will be included until the maximum number of variables allowed in the model is reached.

5.2.6.1 Change to:

- Age and sex
- Co-morbidities
- Co-medication

An adjusted analysis will be conducted with all potential confounders added to the final model. It can only be applied if as a rule of thumb there are at least 10 events per independent variable in the model. If the number of variables in the model would be too large (< 10 events per variable), selection procedure, including only potential confounders that result in a + or - 5% change of the beta-coefficient of the drug exposure of interest when the individual potential confounder is added to an age/gender adjusted model. If this still results in too many variables, only the potential confounders that change this beta-coefficient most will be included until the maximum number of variables allowed in the model is reached.

Model 1:

Null model including age and sex.

Model 2 (Standardized analysis, possible in all databases):

Adjusted model

Model 1 + co-morbidities + co-medication.

Model 3 (Optimal analysis, including all covariates possible for each database):

Adjusted model

Model 2 + variables of the sensitivity analyses

5.2.7 Change from

All analyses will be stratified as follows: “asthma only”, “COPD only”, “asthma and COPD” diagnosis.

Incidence density will be calculated as the number of AMI divided by person-time. Crude incidence density ratios (IDRs) and 95% confidence intervals will be calculated by dividing the incidence density in the current users of LABA by the incidence density in the reference group.

The relative risk stratified by duration of therapy and by subpopulations (i. “asthma only”, ii. “COPD only”, iii. “asthma and COPD”) will be graphically shown with the method proposed by Ramlau-Hansen for inhaled LABA and for the control group (57).

Time-dependent Cox-regression models will be used for confounding factor adjusted analysis. Severity of COPD/asthma and concomitant medication will be handled as time varying covariates. We will calculate hazard ratio for current use of LABA compared to the control group.

5.2.7 Change to:

All analyses will be stratified as follows: “asthma only”, “COPD only”, “asthma and COPD” diagnosis.

In the first and second comparison, past users of inhaled LABA (irrespective of any other drugs) will be compared with recent and current inhaled LABA users, respectively (irrespective of co-medication). In the third analysis, current inhaled LABA users (irrespective of co-medication) will be compared with current Non-LABA-users.

Definition of current Non-LABA users:

A patient is a current user from the beginning of the inhaled SABA or SAMA or LAMA prescription up to the calculated end date of the prescription (this is calculated with the prescribed daily dose or the DDD as surrogate and quantity supplied). Obviously, the real treatment period for patients with SABA or SAMA usage is in clinical practice longer than calculated due to the irregular usage of these reliever medications. This has to be discussed in the publication. If one drug defining non-LABA usage is taken as current usage then the patient will be considered as current usage irrespective of the status of the other medication.

A more detailed analysis (e.g. comparison of current LAMA- versus current LABA-users on a single compound level) will be done in the sensitivity analysis.

Incidence density will be calculated as the number of AMI divided by person-time. Crude incidence density ratios (IDRs) and 95% confidence intervals will be calculated by dividing the incidence density in the past users of LABA by the incidence density in the reference group. (reference group 1: recent inhaled LABA users; reference group 2: current inhaled LABA users). All current inhaled LABA users (irrespective of concomitant treatment) will be compared with all current users within the non-LABA group (comparison 3).

The relative risk stratified by duration of therapy and by subpopulations (i. “asthma only”, ii. “COPD only”, iii. “asthma and COPD”) will be graphically shown with the method proposed by Ramlau-Hansen for inhaled LABA and for the control group (comparisons see above) (57).

Time-dependent Cox-regression models will be used for confounding factor adjusted analysis. Co-morbidities and co-medication will be handled as time varying covariates. We will calculate hazard ratio for ~~current use of~~ **inhaled LABA** compared to the control group (**comparisons see above**).

5.3.5 Change from:

Potential confounders, for which analyses will be adjusted for, are:

- Asthma/COPD disease severity
- Other factors influencing AMI risk

5.3.5 Change to:

Potential confounders, for which analyses will be adjusted for, are:

- co-morbidities
- Co-medication

2.) Sensitivity analysis Change from:

The following drugs (combinations) are defined as potential control groups for the 3 patient strata.

- Inhaled SAMA only (no other drugs for COPD/asthma)
- Inhaled SAMA and ICS (no other drugs for COPD/asthma)
- Inhaled SABA only (no other drugs for COPD/asthma)
- Inhaled SABA and ICS (no other drugs for COPD/asthma)
- Inhaled LAMA only (no other drugs for COPD/asthma)
- Inhaled LAMA and ICS (no other drugs for COPD/asthma)

2.) Sensitivity analysis Change to:

The following drugs (combinations) are defined as potential control groups for the 3 patient strata.

- Inhaled SAMA only (no other drugs for COPD/asthma)
- Inhaled SAMA and ICS (no other drugs for COPD/asthma)
- Inhaled SABA only (no other drugs for COPD/asthma)
- Inhaled SABA and ICS (no other drugs for COPD/asthma)
- Inhaled LAMA only (no other drugs for COPD/asthma)
- Inhaled LAMA and ICS (no other drugs for COPD/asthma)

Exposure of inhaled LAMA will be divided into the same three periods as exposure of inhaled LABA. Inhaled SABA and SAMA are used as reliever medication. So a classification in past and current user is very difficult. For the definition of inhaled SABA/SAMA exposure the DDD per year should be used as surrogate parameter.

8. Change from:

Fourth, the severity of asthma and COPD has to be considered in our analysis as an influence on MI risk cannot be excluded. Unfortunately, severity of these diseases is only rarely depicted in ICD-coding in general and only one database includes lung function parameters (e.g., FEV1). In the most databases in our studies, only the medication regime can be used as a surrogate parameter for the severity

8. Change to:

Fourth, the severity of asthma and COPD has to be considered in our analysis as an influence on MI risk cannot be excluded. Unfortunately, severity of these diseases is only rarely depicted in ICD-coding in general and only one database includes lung function parameters (e.g., FEV1). In the most databases in our studies, only the medication regime can be used as a surrogate parameter for the severity **but due to methodological concerns, treatment steps will be used only in the sensitivity analysis.**

Sensitivity analysis other factors influencing AMI risk Change from:

Some risk factors might have only a minor influence on the AMI risk. These factors will also be considered in the sensitivity analysis (see section “Other risk factors and closely connected diseases (only used in the sensitivity analysis)” and all ATC codes with dosage form “local” in Appendix 2).

Sensitivity analysis Other factors influencing AMI risk Change to:

Some **co-medication and co-morbidities** might have only a minor influence on the AMI risk, these factors will be considered in the sensitivity analysis.

11.2 Amendment 2

Protocol: PROTECT_WP2 Final protocol Beta2_AMI 30 March 2012 with Amendment 1, August 22, 2012

Amendment number: *Nº 2*

Amendments suggested on: 14 January 2013 (see Reasons for amendment)

Amendments finalized on: 14 January 2013

Protocol Owners and Reviewers:

Name	Role
Marietta Rottenkolber ¹	Protocol lead
Sven Schmiedl ³	Protocol backup
Arlene Gallagher ⁴	Protocol reviewer
Xavier Kurz ⁵	Protocol reviewer
Omer de Mol ⁶	Protocol reviewer
Saga Johanson ⁷	Protocol reviewer
Frans Rutten ²	Protocol reviewer
Kourtney Davis ⁸	Protocol reviewer
Ana Sofia Afonso	Protocol reviewer
Marietta Rottenkolber ¹	Database 1 (Bavaria) lead
Joerg Hasford ¹	Database 1 (Bavaria) backup
Miguel Gil ⁹	Database 2 (Bifap) lead

Consuelo Huerta ⁹	Database 2 (Bifap) backup
Ulrik Hesse ¹⁰	Database 3 (DKMA) lead
Frank de Vries ²	Database 3 (DKMA) backup
Paola Primatesta ¹¹	Database 4 (GPRD) lead
Dan Dedman /Jenny Campbell ⁴	Database 4 (GPRD) backup
Eef Voogd ² ,Olaf Klungel ²	Database 5 (Mondriaan) lead
Liset van Dijk ²	Database 5 (Mondriaan) backup
Yolanda Alvarez ⁵	Database 6 (THIN) lead
Ana Ruigomez ¹²	Database 6 (THIN) backup
Mark de Groot ² and Raymond Schlienger ¹¹	WG1 colead
Olaf Klungel ² and Robert Reynolds ¹³	WP2 coleads

1 Institute for Medical Information Sciences, Biometry and Epidemiology, Ludwig-Maximilians-Universitaet Munich, Germany (LMU Muenchen)

2 Universiteit Utrecht, Utrecht, The Netherlands (UU)

3 Philipp Klee-Institute for Clinical Pharmacology, HELIOS Clinic Wuppertal, Wuppertal, Germany / Department of Clinical Pharmacology, School of Medicine, Faculty of Health, Witten/Herdecke University, Germany

4 General Practice Research Database, London, United Kingdom (GPRD)

5 European Medicines Agency, London, United Kingdom (EMA)

6 Genzyme Europe B.V., The Netherlands

7 AstraZeneca AB, Sweden (AZ)

8 GlaxoSmithKline Research and Development LTD, Belgium (GSK)

9 Agencia Espanola de Medicamentos y Productos Sanitarios, Madrid, Spain (AEMPS)

10 Lægemedelstyrelsen (Danish Medicines Agency), Copenhagen, Denmark (DKMA)

11 Novartis Pharma AG, Basel, Switzerland (Novartis)

12 Fundación Centro Español de Investigación Farmacoepidemiológica, Madrid, Spain (CEIFE)

13 Pfizer Ltd, New York, USA (Pfizer)

11.3 Reason(s) for amendment:

This protocol amendment serves to the following purposes:

- e) Clarification of exclusion criteria for the cohort study
- f) Clarification of definitions (e.g., confounder, time dependent analysis, comparisons groups)
- g) Simplification of sensitivity analysis (e.g., reduction in the number of control groups, exposure groups, comparisons)
- h) Deletion of treatment steps as surrogate for severity
- i) Deletion of medication possession ratio / persistence
- j) Minor changes (e.g., deletion of socioeconomic status, further stratification of age groups, further definition of current Non-LABA user)

Protocol Section(s) suggested for amendment

4.1 Data sources

Table 4

Change from:

Study design	Data source	Priority	Partner
Descriptive	Bavaria	High	LMU MUNCHEN

Cohort	Bavaria	High	LMU MUNCHEN
Nested case control	Bavaria	Low	LMU MUNCHEN
Descriptive	BIFAP	High	BIFAP
Nested case control	BIFAP	Low	BIFAP
Descriptive	DKMA	High	DKMA
Cohort	DKMA	Low	DKMA
Descriptive	GPRD	High	Novartis
Cohort	GPRD	High	Novartis
Nested case control	GPRD	Low	Novartis
Descriptive	Mondriaan	High	UU
Cohort	Mondriaan	High	UU
Nested case control	Mondriaan	Low	UU
Case crossover	Mondriaan	Low	UU
Descriptive	THIN	High	EMA
Cohort	THIN	Low	EMA
Nested case control	THIN	Low	EMA
Case crossover	THIN	Low	EMA

Change to:

Study design	Data source	Priority	Partner
Descriptive	Bavaria	High	LMU MUNCHEN
Cohort	Bavaria	High	LMU MUNCHEN
Nested case control	Bavaria	Low	LMU MUNCHEN
Descriptive	BIFAP	High	BIFAP
Nested case control	BIFAP	Low	BIFAP
Descriptive	DKMA	High	DKMA
Cohort	DKMA	Low	DKMA
Descriptive	GPRD	High	Novartis
Cohort	GPRD	High	Novartis
Nested case control	GPRD	Low	Novartis
Descriptive	Mondriaan	High	UU
Cohort	Mondriaan	High	UU
Nested case control	Mondriaan	Low	UU
Case crossover	Mondriaan	Low	UU
Descriptive	THIN	High	EMA
Cohort	THIN	Low	EMA
Nested case control	THIN	Low	EMA
Case crossover	THIN	Low	EMA

4.4 Persistence

Change from:

Several studies pointed out that poor persistence is a relevant problem in the controller/maintenance treatment of asthma and COPD. The medication possession ratio (MPR), which is calculated as the percentage of the treatment time that the patient had drugs available, is a useful marker for estimating the persistence with controller/maintenance medication treatment. In only 8% of patients between the ages of 20 to 29 years, an MPR \geq 80% (reflecting a good persistence) was found using pharmacy data (54). By analyzing data of the Bavarian statutory health insurance physician's association, 65.1% of patients treated for asthma received less than 90 defined daily dose (DDD) for a 1-year-period (48). Since there is some evidence for a dose-related MI risk regarding LABA, we will take into account MPR estimations for these drugs, which are used as controller/maintenance medication.

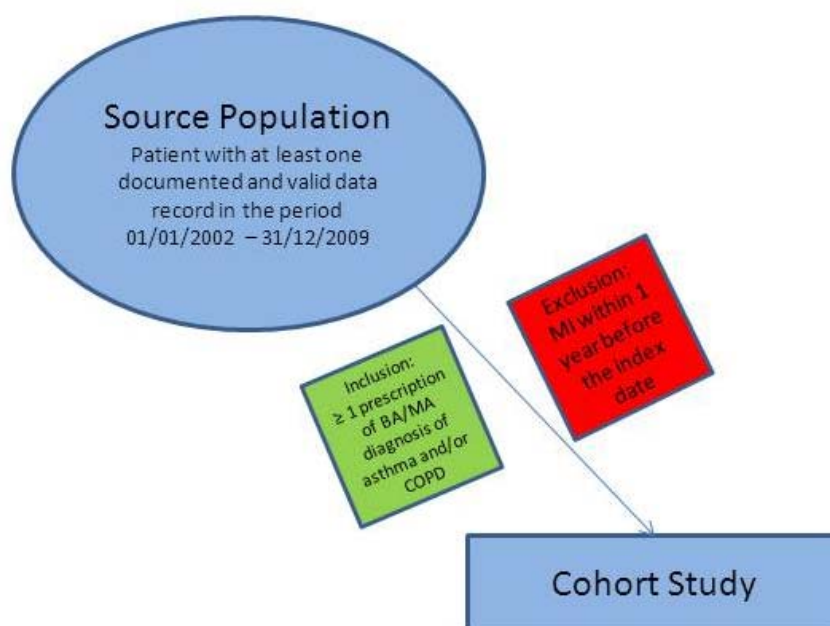
Change to:

~~Several studies pointed out that poor persistence is a relevant problem in the controller/maintenance treatment of asthma and COPD. The medication possession ratio (MPR), which is calculated as the percentage of the treatment time that the patient had drugs available, is a useful marker for estimating the persistence with controller/maintenance medication treatment. In only 8% of patients between the ages of 20 to 29 years, an MPR \geq 80% (reflecting a good persistence) was found using pharmacy data (54). By analyzing data of the Bavarian statutory health insurance physician's association, 65.1% of patients treated for asthma received less than 90 defined daily dose (DDD) for a 1 year period (48). Since there is some evidence for a dose-related MI risk regarding LABA, we will take into account MPR estimations for these drugs, which are used as controller/maintenance medication.~~

5.2.1 Study population

Change from:

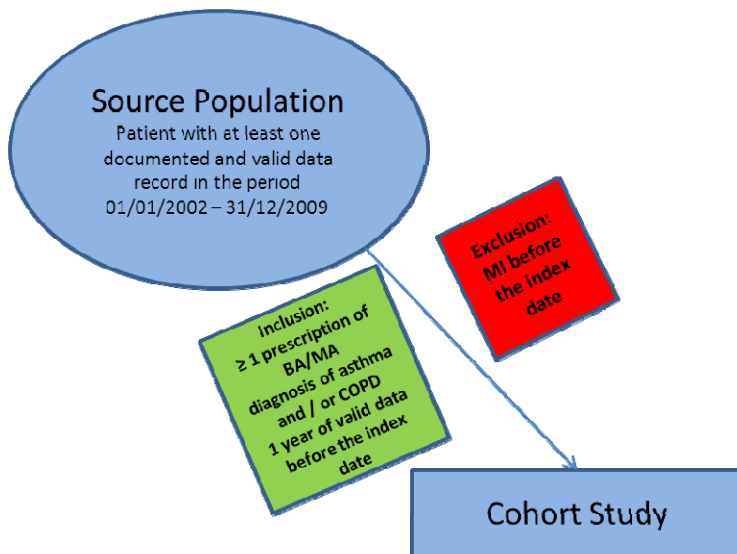
All patients, who received at least one prescription of an inhaled LABA and/or inhaled SABA and/or inhaled LAMA and/or inhaled SAMA and with coded diagnosis of asthma and/or COPD during the study period, will be included in the study. Cohort entry is the first new prescription of an inhaled B2A or an inhaled MA for the patient between January 1st 2002 and December 31st 2009, after one year of valid data with no documented incidence of AMI (this event free year can also be before January 1st 2002, when data is available).



Change to:

All patients, who received at least one prescription of an inhaled LABA and/or inhaled SABA and/or inhaled LAMA and/or inhaled SAMA and with coded diagnosis of asthma and/or COPD during the study period, will be included in the study. Cohort entry is the first new prescription of an inhaled B2A or an inhaled MA for the patient between January 1st 2002 and December 31st 2009 **or latest data available in the database**, after one year of valid data **with no documented incidence of AMI** (this **event-free** year can also be before January 1st 2002, when data is available).

All patients with a previous myocardial infarction will be excluded, as patients who already had a myocardial infarction have elevated risk for another AMI. All patients without a coded diagnosis of asthma or COPD will be excluded from the cohort study.



5.2.3 Definition of exposure

Change from:

The exposure of interest is inhaled LABA use. The duration of inhaled LABA use will be determined by calculating the length of treatment periods. The expected duration of each prescription/dispensing is estimated using the prescribed quantity and the prescribed daily dose. A treatment period of a patient is terminated if more than 91 days occur between the estimated end date of a prescription and the dispensing date of the next prescription. In case of missing data, e.g. the daily dose or package size for the estimation of expected duration, the age-group median duration of use of the specific database will be used.

The total follow-up of the exposure to inhaled LABA will be divided into three periods.

Current user: A patient is a current user from the beginning of the prescription up to the calculated end date of the prescription (this is calculated with the prescribed daily dose and quantity supplied).

Recent user: A patient is a recent user during the 91 days following the calculated end date of the prescription.

Past user: A patient is a past user after 91 days following the calculated end of prescription. The period of “Past user” will expire if the patient becomes a new user or on the end of follow-up.

Standard prescription durations are different for each database (and thus country). 91 days is chosen as standard in this study. Other durations are examined in a sensitivity analysis.

A patient can switch between current/recent and past periods and between the treatment classes. If a patient switches between the treatment classes a new treatment period starts at the date of the prescription of the new drug.

Current use will be stratified to the following parameters:

Duration: the duration is the sum of the consecutive periods of LABA prescription (categories 0-3 month, 3-6 month, more than 6 month)

Persistence: as surrogate parameter for persistence the medication possession ratio will be used (categories <0.8 and ≥0.8)

Change to:

The exposure of interest is inhaled LABA use. The duration of inhaled LABA use will be determined by calculating the length of treatment periods. The expected duration of each prescription/dispensing is estimated using the prescribed quantity and the prescribed daily dose. A treatment period of a patient is terminated if more than 91 days occur between the estimated end date of a prescription and the dispensing date of the next prescription. In case of missing data, e.g. the daily dose or package size for the estimation of expected duration, the age-group median duration of use of the specific database will be used. **If no age-group median treatment duration can be calculated, a treatment duration of 90 days is used as an assumption. The daily dose and the quantity supplied are often unknown in both Mondriaan databases (NPCRD and AHC), so a time period of 90 days will be used as a surrogate in both databases, due to the low reliability of the information available**

If a patient has two prescriptions of the same drug class or the same drug on the same day, the respective treatment periods are summed. If the same drug class or drug is prescribed on different days, treatment duration will be calculated starting at the most recent prescription (no summation of treatment durations).

The total follow-up of the exposure to inhaled LABA will be divided into three periods.

Current user: A patient is a current user from the beginning of the prescription up to the calculated end date of the prescription ~~(this is calculated with the prescribed daily dose and quantity supplied).~~

Recent user: A patient is a recent user during the 91 days following the calculated end date of the prescription.

Past user: A patient is a past user after 91 days following the calculated end of prescription. The period of "Past user" will expire if the patient becomes a new user or on the end of follow-up.

Standard prescription durations are different for each database (and thus country). 91 days is chosen as standard in this study. Other durations are examined in a sensitivity analysis.

A patient can switch between current/recent and past periods and between the treatment classes. If a patient switches between the treatment classes a new treatment period starts at the date of the prescription of the new drug **(see also chapter 3.6.3).**

Current use will be stratified to the following parameters:

Duration: the duration is the sum of the consecutive periods of LABA prescription **(0-14 days (0-2 weeks), 14-42 days (>2 – 6 weeks), >42 days (> 6 weeks)categories 0-3 month, 3-6 month, more than 6 month)**

~~Persistence: as surrogate parameter for persistence the medication possession ratio will be used (categories <0.8 and ≥0.8)~~

5.2.4 Definition of outcome

Change from:

The outcome of the study is the first AMI in the study period. AMI is coded in the International Classification of Diseases version 10 (ICD10) as I21.- (AMI). In GPRD and THIN adequate codes read codes will be applied. In Mondriaan and BIFAP, the international classification of primary care (ICPC) code K75: AMI will be used. In sensitivity analyses we will also distinguish between fatal and non-fatal AMI for those databases that allow this distinction.

Change to:

The outcome of the study is the first **ever** AMI in the study period. AMI is coded in the International Classification of Diseases version 10 (ICD10) as I21.- (AMI). In GPRD and THIN adequate codes read codes will be applied. In Mondriaan and BIFAP, the international classification of primary care (ICPC) code K75: AMI will be used. In sensitivity analyses we will also distinguish between fatal and non-fatal AMI for those databases that allow this distinction.

5.2.5 Change of indication (switching problem)

Change from:

Since patients with a diagnosis of asthma may develop COPD within the study period (and vice versa), we have to reproduce this problem in our analysis. Nevertheless, indication for each prescription is documented in some databases only and therefore, a static assessment of indication will be performed as described in section 5.1.3.2. Within the sensitivity analysis, a dynamic assessment of indication will be performed.

Change to:

Since patients with a diagnosis of asthma may develop COPD within the study period (and vice versa), we have to reproduce this problem in our analysis. Nevertheless, indication for each prescription is documented in some databases only and therefore, a static assessment of indication will be performed as described in section 5.1.3.2. **For defining the indication, longest available period documented in the respective databases (life-time period, if possible) should be considered.** Within the sensitivity analysis, a dynamic assessment of indication will be performed. **Additionally, sequence of diseases will be analysed for the COPD-and-asthma stratum (number of patients diagnosed with asthma followed by a COPD diagnosis and vice versa).**

5.2.6.1 Potential confounders, for which analyses will be adjusted for, are:

Change from:

- Age and sex
- Co-morbidities
- Co-medication

An adjusted analysis will be conducted with all potential confounders added to the final model. It can only be applied if as a rule of thumb there are at least 10 events per independent variable in the model. If the number of variables in the model would be too large (< 10 events per variable), selection procedure, including only potential confounders that result in a + or - 5% change of the beta-coefficient of the drug exposure of interest when the individual potential confounder is added to an age/gender adjusted model. If this still results in too many variables, only the potential confounders that change this beta-coefficient most will be included until the maximum number of variables allowed in the model is reached.

Model 1:

Null model including age and sex.

Model 2 (Standardized analysis, possible in all databases):

Adjusted model

Model 1 + co-morbidities + co-medication.

Model 3 (Optimal analysis, including all covariates possible for each database):

Adjusted model

Model 2 + variables of the sensitivity analyses

Change to:

- Age and sex
- Co-morbidities
- Co-medication

An adjusted analysis will be conducted with all potential confounders added to the final model. It can only be applied if as a rule of thumb there are at least 10 events per independent variable in the model. If the number of variables in the model would be too large (< 10 events per variable), selection procedure, including only potential confounders that result in a + or - 5% change of the beta-coefficient of the drug exposure of interest when the individual potential confounder is added to an age/gender adjusted model. If this still results in too many variables, only the potential confounders that change this beta-coefficient most will be included until the maximum number of variables allowed in the model is reached.

Model 1 (DSD Table 5):

Null model including age and sex.

Model 2 (Standardized analysis, possible in all databases, DSD Table 6-8):

Adjusted model

Model 1 + co-morbidities + co-medication.

Model 3 (Optimal analysis, including all covariates possible for each database, DSD Table 9-13):

Adjusted model

Model 2 + variables of the sensitivity analyses

Assessment of co-morbidities and co-medication for model 2 and 3

Assessment of co-morbidities and co-medication: every sixth months and in the case of exposure changes from one year prior to study entry until the end of the study period

Co-morbidities: from the date a patient is diagnosed with a particular disease, this disease will be considered as present disease for the whole study period onwards

Co-medication: presence of co-medication will be assessed every sixth months and a patient can switch between use and non-use in contrast to co-morbidities

5.2.6.2 Other factors influencing AMI risk (Appendix 2)

Change from:

Many pre-existing co-morbidities (e.g. diagnosed and/or treated ischemic heart disease, arrhythmias, diabetes, hyperlipidemia) and life style (e.g. obesity, smoking) can increase the risk for myocardial infarction and for some drugs, an increased risk cannot be excluded (e.g. COX-II inhibitors). From a drug perspective, some drugs (e.g. ASS, lipid lowering drugs, and antidiabetic drugs) can also be used in defining an AMI risk population (see above).

Change to:

~~Many pre-existing co-morbidities (e.g. diagnosed and/or treated ischemic heart disease, arrhythmias, diabetes, hyperlipidemia) and life style (e.g. obesity, smoking) can increase the risk for myocardial infarction and for some drugs, an increased risk cannot be excluded (e.g. COX-II inhibitors). From a drug perspective, some drugs (e.g. ASS, lipid lowering drugs, and antidiabetic drugs) can also be used in defining an AMI risk population (see above).~~

5.2.7 Analysis

Change from:

All analyses will be stratified as follows: “asthma only”, “COPD only”, “asthma and COPD” diagnosis.

In the first and second comparison, past users of inhaled LABA (irrespective of any other drugs) will be compared with recent and current inhaled LABA users, respectively (irrespective of co-medication). In the third analysis, current inhaled LABA users (irrespective of co-medication) will be compared with current Non-LABA-users.

Definition of current Non-LABA users:

A patient is a current user from the beginning of the inhaled SABA or SAMA or LAMA prescription up to the calculated end date of the prescription (this is calculated with the prescribed daily dose or the DDD as surrogate and quantity supplied). Obviously, the real treatment period for patients with SABA or SAMA usage is in clinical practice longer than calculated due to the irregular usage of these reliever medications. This has to be discussed in the publication. If one drug defining non-LABA usage is taken as current usage then the patient will be considered as current usage irrespective of the status of the other medication.

A more detailed analysis (e.g. comparison of current LAMA- versus current LABA-users on a single compound level) will be done in the sensitivity analysis.

Incidence density will be calculated as the number of AMI divided by person-time. Crude incidence density ratios (IDRs) and 95% confidence intervals will be calculated by dividing the incidence density in the past users of LABA by the incidence density in the reference group. (reference group 1: recent inhaled LABA users; reference group 2: current inhaled LABA users). All current inhaled LABA users (irrespective of concomitant treatment) will be compared with all current users within the non-LABA group (comparison 3).

The relative risk stratified by duration of therapy and by subpopulations (i. “asthma only”, ii. “COPD only”, iii. “asthma and COPD”) will be graphically shown with the method proposed by Ramlau-Hansen for inhaled LABA and for the control group (comparisons see above) (57).

Time-dependent Cox-regression models will be used for confounding factor adjusted analysis. Co-morbidities and co-medication will be handled as time varying covariates. We will calculate hazard ratio for inhaled LABA compared to the control group (comparisons see above).

Change to:

All analyses will be stratified as follows: “asthma only”, “COPD only”, “asthma and COPD” diagnosis.

In the first ~~and second~~ comparison, ~~past~~ current users of inhaled LABA (irrespective of any other drugs) will be compared with ~~recent and~~ current inhaled Non-LABA users, ~~respectively (irrespective of co-medication)~~. In the ~~second and~~ third analysis, current inhaled LABA users (irrespective of co-medication) will be compared with ~~recent or past LABA users, respectively (irrespective of co-medication)~~ Non-LABA users.

Definition of current Non-LABA users:

A patient is a current user from the beginning of the inhaled SABA or SAMA or LAMA prescription up to the calculated end date of the prescription (this is calculated with the prescribed daily dose or the DDD as surrogate and quantity supplied). ~~Treatment periods will be calculated according to LABA.~~ Obviously, the real treatment period for patients with SABA or SAMA usage is in clinical practice longer than calculated due to the irregular usage of these reliever medications. ~~Thus, different lengths for the treatment period will be evaluated in the sensitivity analysis (180 days). After the respective treatment period, patients become (in absence of LABA usage) a non-current Non-LABA-user (no “recent” and no “past” category for Non-LABA users). This has to be discussed in the publication.~~ If one drug defining non-LABA usage is taken as current usage then the patient will be considered as current usage irrespective of the status of the other medication.

A more detailed analysis (e.g. comparison of current LAMA- versus current LABA-users on a single compound level) will be done in the sensitivity analysis.

~~Switches between LABA and Non-LABA~~

~~A patient can switch between current, recent and past periods and between the treatment classes [LABA vs. Non-LABA]. If a patient switches between the treatment classes a new treatment period starts at the date of the prescription of the new drug. As described in the protocol, LABA group is defined irrespective of any other (Non-LABA) drug whereas Non-LABA group is defined by usage of SABA, SAMA or LAMA but not using LABA. So the following switches are possible:~~

- ~~• current LABA -> recent LABA~~
- ~~• recent LABA -> past LABA~~
- ~~• past LABA -> current LABA~~
- ~~• recent LABA -> current LABA~~
- ~~• current Non-LABA -> current LABA~~
- ~~• current Non-LABA -> non-current Non-LABA~~
- ~~• non-current Non-LABA -> current Non-LABA~~
- ~~• non-current Non-LABA -> current LABA~~

Incidence density will be calculated as the number of AMI divided by person-time. Crude incidence density ratios (IDRs) and 95% confidence intervals will be calculated by dividing the incidence density in the ~~past current~~ users of inhaled LABA by the incidence density in the reference group, (reference group 1: ~~current inhaled Non-LABA users~~ recent inhaled LABA users; reference group 2: ~~recent inhaled LABA users,~~ reference group 3: ~~past inhaled LABA users~~ current inhaled LABA users). ~~All current inhaled LABA users (irrespective of concomitant treatment) will be compared with all current users within the non-LABA group (comparison 3).~~

The relative risk stratified by duration of therapy and by subpopulations (i. “asthma only”, ii. “COPD only”, iii. “asthma and COPD”) will be graphically shown with the method proposed by Ramlau-Hansen for inhaled LABA and for the control group (comparisons see above) (57).

Time-dependent Cox-regression models will be used for confounding factor adjusted analysis. Co-morbidities and co-medication will be handled as time varying covariates (see 5.2.6.1). We will calculate hazard ratio for inhaled LABA compared to the control group (comparisons see above).

6 Sensitivity analyses (optional)

Change from:

To investigate the influence of several factors important in this association, the following sensitivity analyses are proposed.

Change to:

To investigate the influence of several factors important in this association, the following sensitivity analyses are proposed. Within these analyses, the following issues are of high importance (additional stratification of exposure, additional stratification of control groups, oral and i.v. formulations (included as confounders), additional drugs as surrogates for severity). Other issues (additional definition of current, recent, and past use, dynamic assessment of indication, discrimination between fatal and non-fatal AMI, additional co-medication (included as confounders) and discrimination between incident and non-incident current LABA users are of minor importance only.

6.1 Further stratification of exposure

Change from:

Since the two inhaled LABA compounds differ with regard to some pharmacokinetic aspects (e.g. onset of bronchodilative effect is much faster for formoterol compared to salmeterol [see introduction]), we will evaluate not only inhaled LABA as whole but also the two compounds separately. As shown by SMART results (10), concomitant usage of ICS in patients receiving salmeterol is crucial and may influence overall mortality significantly. Therefore, we will also assess both inhaled LABA compounds with regard to the concomitant usage of ICS (formoterol with and without ICS/salmeterol with and without ICS). Patients with switches between treatment groups will be counted for each group.

- Formoterol only (no other drugs for COPD/asthma)
- Salmeterol only (no other drugs for COPD/asthma)
- Formoterol and concomitant ICS fixed combination / one inhaler (no other drugs for COPD/asthma)
- Salmeterol and concomitant ICS fixed combination / one inhaler (no other drugs for COPD/asthma)
- Formoterol and concomitant ICS dispensed in two inhalers (no other drugs for COPD/asthma)
- Salmeterol and concomitant ICS dispensed in two inhalers (no other drugs for COPD/asthma)
- Other combinations including Formoterol
- Other combinations including Salmeterol

Change to:

6.1 Additional ~~Further~~ stratification of exposure (high priority)

Since the two inhaled LABA compounds differ with regard to some pharmacokinetic aspects (e.g. onset of bronchodilative effect is much faster for formoterol compared to salmeterol [see introduction]), we will evaluate not only inhaled LABA as whole but also the two compounds separately. As shown by SMART results (10), concomitant usage of ICS in patients receiving salmeterol is crucial and may influence overall mortality significantly. Therefore, we will also assess both inhaled LABA compounds with regard to the concomitant usage of ICS (formoterol with and without ICS/salmeterol with and without ICS). Patients with switches between treatment groups will be counted for each group.

- Inhaled LABA (formoterol or salmeterol) (without ICS but irrespective of any other drugs for COPD/asthma)
- Inhaled LABA (formoterol or salmeterol) and concomitant ICS (fixed combinations)
- Inhaled LABA (formoterol or salmeterol) and concomitant ICS dispensed in two inhalers.

~~Formoterol only (no other drugs for COPD/asthma)~~

~~Salmeterol only (no other drugs for COPD/asthma)~~

~~Formoterol and concomitant ICS fixed combination / one inhaler (no other drugs for COPD/asthma)~~

~~Salmeterol and concomitant ICS fixed combination / one inhaler (no other drugs for COPD/asthma)~~

~~Formoterol and concomitant ICS dispensed in two inhalers (no other drugs for COPD/asthma)~~

~~Salmeterol and concomitant ICS dispensed in two inhalers (no other drugs for COPD/asthma)~~

~~Other combinations including Formoterol~~

~~Other combinations including Salmeterol~~

6.2 Further definition of control groups

Change from:

The following drugs (combinations) are defined as potential control groups for the 3 patient strata.

- Inhaled SAMA only (no other drugs for COPD/asthma)
- Inhaled SAMA and ICS (no other drugs for COPD/asthma)
- Inhaled SABA only (no other drugs for COPD/asthma)
- Inhaled SABA and ICS (no other drugs for COPD/asthma)
- Inhaled LAMA only (no other drugs for COPD/asthma)
- Inhaled LAMA and ICS (no other drugs for COPD/asthma)

Exposure of inhaled LAMA will be divided into the same three periods as exposure of inhaled LABA. Inhaled SABA and SAMA are used as reliever medication. So a classification in past and current user is very difficult. For the definition of inhaled SABA/SAMA exposure the DDD per year should be used as surrogate parameter.

Change to:

6.2 Additional ~~Further~~ definitions of control groups (high priority)

The following drugs (combinations) are defined as potential control groups for the 3 patient strata.

- Inhaled SAMA only or inhaled SAMA and ICS (no other drugs for COPD/asthma)
- Inhaled SABA only or inhaled SABA and ICS (no other drugs for COPD/asthma)

- Inhaled LAMA only or inhaled LAMA and ICS (no other drugs for COPD/asthma)

~~Inhaled SAMA only (no other drugs for COPD/asthma)~~

~~Inhaled SAMA and ICS (no other drugs for COPD/asthma)~~

~~Inhaled SABA only (no other drugs for COPD/asthma)~~

~~Inhaled SABA and ICS (no other drugs for COPD/asthma)~~

~~Inhaled LAMA only (no other drugs for COPD/asthma)~~

~~Inhaled LAMA and ICS (no other drugs for COPD/asthma)~~

Exposure of inhaled LAMA will be divided into the same three periods as exposure of inhaled LABA. Inhaled SABA and SAMA are used as reliever medication. So a classification in past and current user is very difficult. For the definition of inhaled SABA/SAMA exposure the DDD per year should be used as surrogate parameter.

6.2.1 Additional comparisons to be made taking into account additional stratifications of exposure and additional definitions of control group as stated above (high priority):

- Current inhaled LABA (formoterol and salmeterol, without ICS but irrespective of any other drugs for COPD/asthma) vs. current Inhaled LABA (formoterol or salmeterol) and concomitant ICS in a fixed combination
- Current inhaled LABA (without ICS but irrespective of any other drugs for COPD/asthma) vs. current Inhaled LABA (formoterol or salmeterol) and concomitant ICS dispensed in two inhalers
- Current Inhaled LABA (formoterol or salmeterol) and concomitant ICS fixed combinations vs. current Inhaled LABA (formoterol or salmeterol) and concomitant ICS dispensed in two inhalers
- Current Inhaled LABA (formoterol or salmeterol) or current inhaled LABA (formoterol or salmeterol) and concomitant ICS fixed combinations or current inhaled LABA (formoterol or salmeterol) and concomitant ICS dispensed in two inhalers vs. current inhaled SAMA only or inhaled SAMA and ICS (no other drugs for COPD/asthma)
- Current Inhaled LABA (formoterol or salmeterol) or current inhaled LABA (formoterol or salmeterol) and concomitant ICS fixed combinations or current inhaled LABA (formoterol or salmeterol) and concomitant ICS dispensed in two inhalers vs. current inhaled SABA only or inhaled SABA and ICS (no other drugs for COPD/asthma)
- Current Inhaled LABA (formoterol or salmeterol) or current inhaled LABA (formoterol or salmeterol) and concomitant ICS fixed combinations or current inhaled LABA (formoterol or salmeterol) and concomitant ICS dispensed in two inhalers vs. current inhaled LAMA only or inhaled LAMA and ICS (no other drugs for COPD/asthma)
- Current Inhaled formoterol vs current inhaled salmeterol

6.3 Oral and i.v. formulation (high importance)

Formulation with a higher systemic exposure (oral, intravenous application) will be considered as confounders.

6.3 Further definition of **current**, recent and past use

Change from:

The duration of the recent and past use will be varied as follows:

Current use	Recent use	Past use
-------------	------------	----------

from the beginning of the prescription up to the calculated end date of the prescription	60 days after the calculated end date	More than 60 days after the calculated end date
from the beginning of the prescription up to the calculated end date of the prescription	30 days after the calculated end date	More than 30 days after the calculated end date

Change to:

6.3 Further definition of current, recent and past use (low priority)

The duration of the LABA recent and LABA past use will be varied as follows:

Current use	Recent use	Past use
from the beginning of the prescription up to the calculated end date of the prescription	60 days after the calculated end date	More than 60 days after the calculated end date
from the beginning of the prescription up to the calculated end date of the prescription	30 days after the calculated end date	More than 30 days after the calculated end date

The duration of the Non-LABA recent use will be varied as follows:

Current use	Non-Current user
from the beginning of the prescription up to 180 days	180 days after beginning of the prescription

6.4 Further definitions of age groups

Change from:

The upper age group is now identified as 80+. It is expected that the number of patients above 80 are low. This has statistical implications, as groups of patients can become too small to analyse. As age is an important factor in the risk of AMI, the assignment of one large 80+ group can influence the result of the study. In a sensitivity analysis, the influence on the definition of the upper age group will be investigated. Therefore, the following age group will be analysed:

- i) 80-90, 90+

Change to:

~~The upper age group is now identified as 80+. It is expected that the number of patients above 80 are low. This has statistical implications, as groups of patients can become too small to analyse. As age is an important factor in the risk of AMI, the assignment of one large 80+ group can influence the result of the study. In a sensitivity analysis, the influence on the definition of the upper age group will be investigated. Therefore, the following age group will be analysed:~~

- ~~i) 80-90, 90+~~

6.5 Fatal vs. non-fatal AMI (low priority)

Change from:

6.5 Fatal vs. non-fatal AMI

Change to:

6.5 Fatal vs. non-fatal AMI (low priority)

6.6 Dynamic assessment of indication

Change from:

Since patients with a diagnosis of asthma may develop COPD within the study period (and vice versa), we have to reproduce this problem in our analysis. Nevertheless, indication for each prescription is documented in some databases only and therefore, a static assessment of indication will be performed in the descriptive studies and in the cohort studies.

In the sensitivity analysis, a dynamic assessment of indication will be performed every three months for the cohort studies (see figure 4). If a patient has been coded with asthma first and develops COPD afterwards (within the study period), the patient will be grouped before the diagnosis of COPD as an “asthma” patient and starting with the first COPD diagnosis as “asthma and COPD” patient (irrespective whether both diagnoses are coded from that point of time). Same procedure will be performed vice versa (patient with a coded COPD developing asthma). Our approach will result in three patients’ strata which might include the same patients in two strata. Therefore, “asthma only” and the “asthma and COPD” cohort as well as the “COPD” and the “asthma and COPD” cohort will not be fully independent. On the other hand, the cohorts of “asthma only” and “COPD only” patients are fully independent.

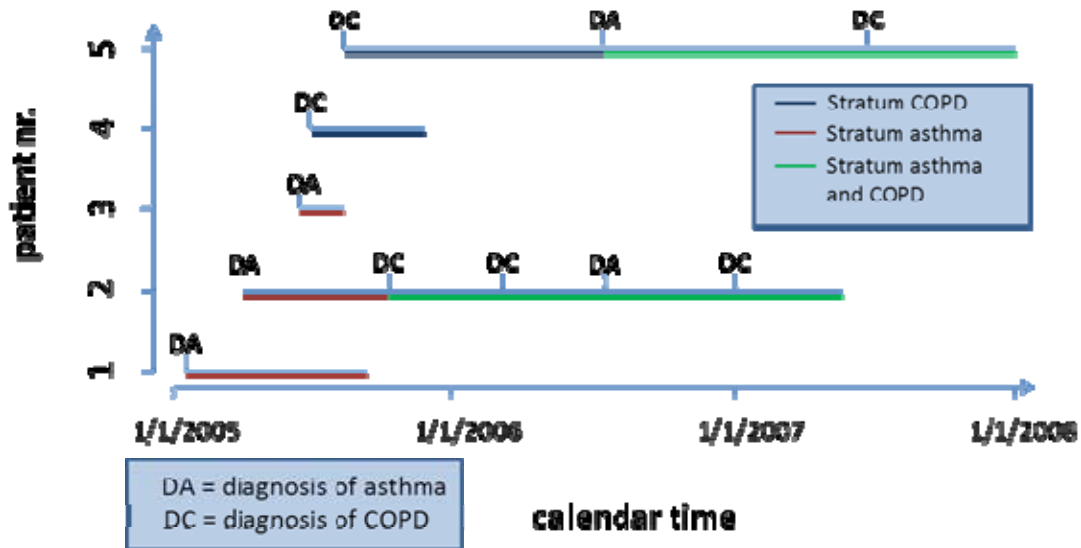


Figure 4: Change of indication: dynamic assessment

Change to:

6.6 Dynamic assessment of indication (low priority)

Since patients with a diagnosis of asthma may develop COPD within the study period (and vice versa), we have to reproduce this problem in our analysis. Nevertheless, indication for each prescription is documented in some databases only and therefore, a static assessment of indication will be performed in the descriptive

studies and in the cohort studies. For defining the indication, longest available period documented in the respective databases (life-time period, if possible) should be considered.

In the sensitivity analysis, a dynamic assessment of indication will be performed every three months for the cohort studies (see figure 4). If a patient has been coded with asthma first and develops COPD afterwards (within the study period), the patient will be grouped before the diagnosis of COPD as an “asthma” patient and starting with the first COPD diagnosis as “asthma and COPD” patient (irrespective whether both diagnoses are coded from that point of time). Same procedure will be performed vice versa (patient with a coded COPD developing asthma). Our approach will result in three patients’ strata which might include the same patients in two strata. Therefore, “asthma only” and the “asthma and COPD” cohort as well as the “COPD” and the “asthma and COPD” cohort will not be fully independent. On the other hand, the cohorts of “asthma only” and “COPD only” patients are fully independent.

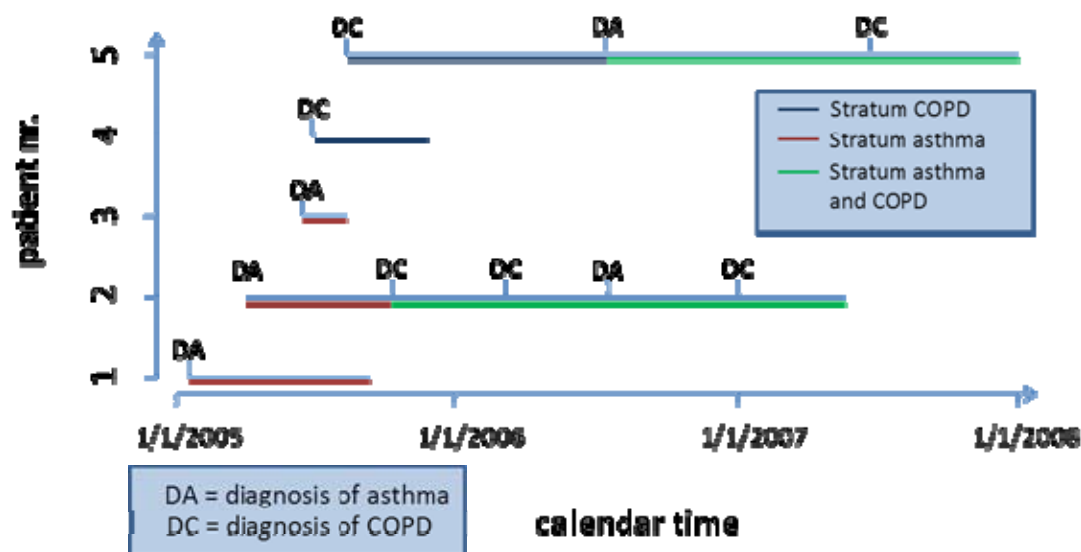


Figure 4: Change of indication: dynamic assessment

Additionally, sequence of diseases will be analysed for the COPD-and-asthma stratum (number of patients diagnosed with asthma followed by a COPD diagnosis and vice versa).

6.7 Severity

Change from:

Since an influence on MI risk cannot be excluded, the severity of asthma and COPD has to be included in our analysis as a confounding factor (59). Unfortunately, severity of both diseases is only rarely depicted in ICD-coding in general. Furthermore, clinically relevant parameters describing disease severity (e.g. lung function parameters, number of hospitalisations) are not documented in most databases. Treatment steps could be useful for defining disease severity but since LABA is used in most treatment steps (particular in asthma patients), methodological aspects limit the results to a huge extent. Therefore, treatment steps will be used in the sensitivity analysis only (8).

Regarding the numerous drugs used in COPD and asthma and possible drug combinations, treatment steps can only be judged as a very rough surrogate for disease severity. Additionally, drugs not primarily recommended in the guidelines (and depicted in the treatment steps) will also be used (e.g. theophylline in COPD patients). Therefore we included the group “combinations not listed” in our treatment steps. Furthermore, prescription behaviour might have changed within the last years affecting the study population (e.g. LABA usage without ICS PROTECT_WP2 Final Protocol Beta2_AMI 30 March 2012

has been re-assessed after the SMART trial (10)). Therefore, we did change the current asthma treatment step 3 consisting of a fixed combination of LABA/ICS in “LABA or LABA/low dose ICS”. In summary, there are several severe limitations regarding the usage of treatment steps for assessing disease severity.

Furthermore, focussing on drugs only and non-considering of clinical issues can only give a limited impression of severity as stated also in the GINA guidelines: “It is important to recognize, however, that asthma severity involves both the severity of the underlying disease and its responsiveness to treatment. Thus, asthma could present with severe symptoms and airflow obstruction, but become completely controlled with low-dose treatment”. Regarding the relevance of clinical endpoints we want to use clinical parameters (if available) to refine disease severity assessment in the sensitivity analysis for asthma and COPD patients separately. For the other patients included in our study (COPD & asthma) relevant clinical issues (e.g. hospitalisation) will also be defined.

Since severity will change over time, we will (re)assess severity regarding 3-months intervals. Additionally, treatment steps according to the guidelines can be used only in patients with a diagnosis of asthma or COPD. For patients suffering from both diseases (our 3rd cohort) simplified treatment steps will be used for defining severity.

6.7.1.1 Definition of treatment steps for asthma:

According to recent guidelines published by the Global Initiative for Asthma ([GINA] see figure 4 (1)), drugs can be used for defining treatment steps. In general, asthma (and COPD) drugs can be divided in reliever compounds used as needed medication due to a rapid bronchodilation and in controller medication which is used on a regular base (several long-acting, slow-acting mechanisms, e.g. anti-inflammation). In treatment step 1, reliever medication only is given to the patient but in treatment step 2, controller medication is started (and reliever medication is added on an “as needed base” by the patient).

		← Reduce			Treatment Steps		Increase →		
		Step 1	Step 2	Step 3	Step 4	Step 5			
		Asthma education Environmental control							
		As needed rapid-acting β ₂ -agonist	As needed rapid-acting β ₂ -agonist						
Controller options			Select one	Select one	Add one or more	Add one or both			
			Low-dose inhaled ICS [†]	Low-dose ICS plus long-acting β ₂ -agonist	Medium-or high-dose ICS plus long-acting β ₂ -agonist	Oral glucocorticosteroid (lowest dose)			
			Leukotriene modifier [‡]	Medium-or high-dose ICS	Leukotriene modifier	Anti-IgE treatment			
				Low-dose ICS plus leukotriene modifier	Sustained release theophylline				
				Low-dose ICS plus sustained release theophylline					

[†] Inhaled glucocorticosteroids. [‡] Receptor antagonist or synthesis inhibitors.

Figure 4: Asthma treatment according to the GINA guidelines (preferred controller options are shown in shadowed boxes; figure taken from Hoshino et al. (60) Please note: Whereas “rapid-acting” B2A are stated as “as-needed” medication the GINA guidelines (1), “short-acting” B2A are stated as “as-needed” medication in the British Guideline (4) (see figure 5).

Whereas in some guidelines (e.g. GINA (1)) RABA (rapid acting B2A) are stated as reliever medication in other guidelines (e.g. British guideline on the Management of Asthma (4), see figure 5) SABA are recommended.

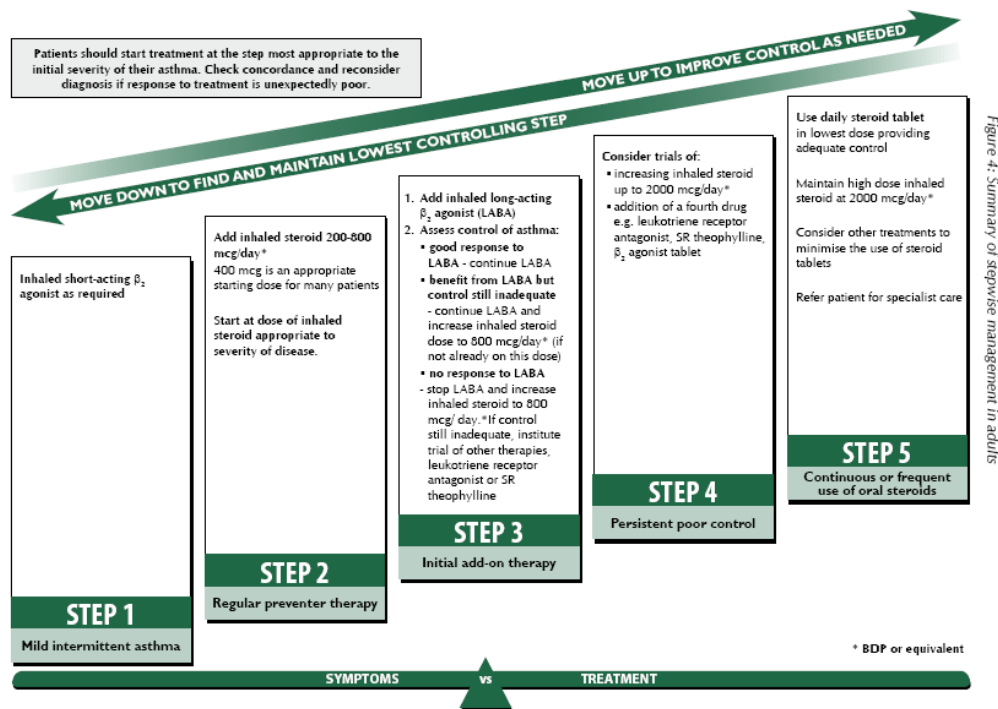


Figure 5: Asthma treatment according to the British Guideline on the Management of Asthma (May 2008, revised May 2011(4)). Please note: Whereas “short-acting” B2A are stated as “as-needed” medication in the British Guideline, “rapid-acting” B2A are stated as “as-needed” medication the GINA guidelines (1) (see figure 4).

As discussed above, formoterol is a rapid-acting LABA (and a member of the RABA class) and might be used in treatment step 1 as reliever medication (see e.g. GINA (1)). On the other hand, formoterol should be used - as already discussed for LABAs and according to the guidelines – in combination with ICS only. Focussing on the effect duration (and the combined usage of LABA and ICS which is widely stated as treatment step 3), we decided to use the conventional classification system (SABA and LABA) as stated e.g. in the recently published British guideline on the Management of Asthma (4) (see figure 5). Thus, we want to reduce confusion regarding formoterol and all patients using this compound will be included in our analysis as at least treatment step 3.

Treatment step 1*: Reliever medication only: SABA, SAMA, short-acting theophylline

Treatment step 2: Low dose ICS or leukotriene modifier (plus reliever medication)

Treatment step 3: LABA or LABA/low dose ICS or medium /high-dose ICS or low-dose ICS plus leukotriene modifier or low-dose ICS plus sustained release theophylline (plus reliever medication)

Treatment step 4: Treatment step 3 plus one or more of the following: LABA and medium- or high-dose ICS and/or leukotriene modifier and/or sustained release theophylline

Treatment step 5**: Addition of oral glucocorticosteroid to Step 4

Treatment step 6**: Addition of omalizumab to step 5

Treatment step 7: combinations not listed above

*Since inhaled SABA is the preferred drug class within the reliever medication group and other reliever drug (classes) e.g. oral SABA and theophylline are typically used in patients with a more severe asthma due to the increased systemic drug exposure, treatment step 1 will be split in two groups (inhaled, oral reliever medication) which will also be considered for the other treatment steps in the sensitivity analysis.

**Since omalizumab should only be used as a “last” option, step 5 according to GINA has been divided in two steps (chronic oral corticosteroids [duration of prescription at least 30 days] and omalizumab).

As stated above, time course of severity changes has to be taken into account. Since seasonal influences are well-known particularly for extrinsic (allergic) asthma, a higher asthma severity during spring and summer may occur but in autumn / winter, asthma medication might be reduced. Due to these changes, severity assessment of asthma patients will be performed on a 3-months interval (quarter).

6.7.1.2 Definition of treatment steps for COPD

Using a current guideline of the Global initiative for chronic Obstructive Lung Disease (2) (see figure 6), drug therapy can also be used for a rough estimation of the severity of COPD. As in asthma patients treatment of COPD is started using reliever medication “as needed” and by reaching treatment step 2, maintenance medication is given on a regular base (and reliever medication as needed).

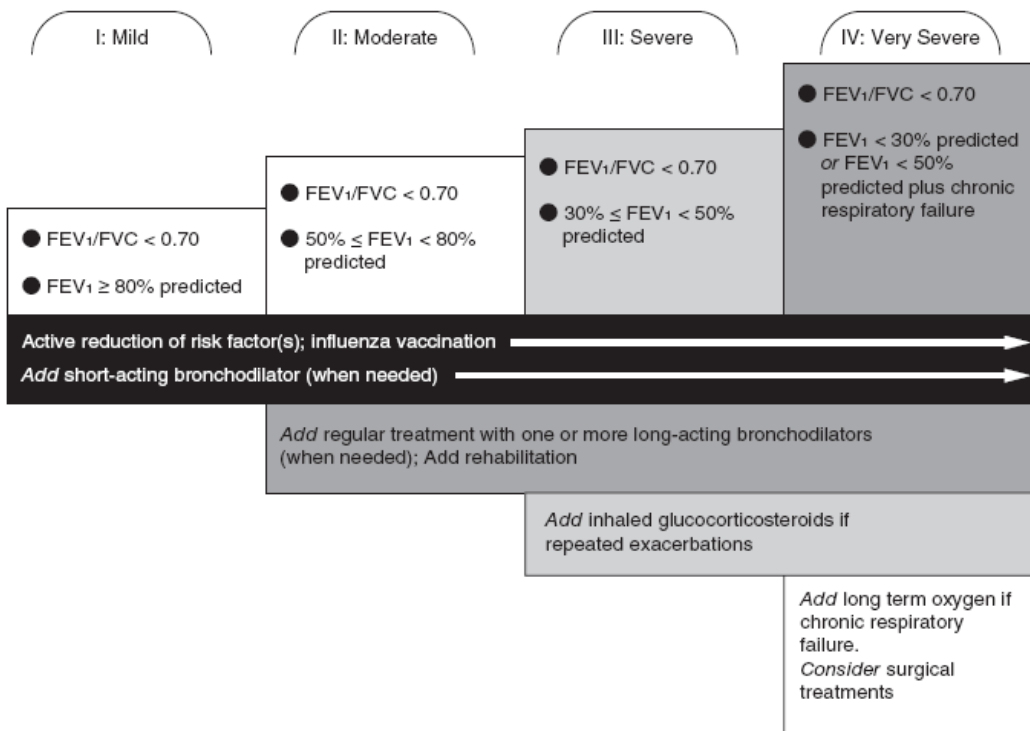


Figure 6: COPD treatment according to GOLD (figure taken from Hoshino et al. (60))

Treatment step 1*: Reliever medication only: SABA, SAMA, short-acting theophylline

Treatment step 2: LAMA or LABA (plus reliever medication)

Treatment step 3: Treatment step 2 plus inhaled corticosteroids

Treatment step 4: Addition of systemic corticosteroids (usually just for acute exacerbations)

Treatment step 5: combinations not listed above (e.g. slow-release theophylline)

*Whereas inhaled reliever medication is stated as preferred treatment, other reliever drug (classes) e.g. oral SAMA, SABA and theophylline are typically used in patients with a more severe COPD. Due to the increased systemic drug exposure, treatment step 1 will be split in two groups (inhaled, oral reliever medication) which will also be considered for the other treatment steps in the sensitivity analysis.

Since non-drug related issues are documented in some databases only, we will use oxygen therapy and surgical interventions defining very severe COPD in the sensitivity analysis only.

As for asthma, time change of severity steps has to be taken into account. From a pathophysiological perspective, COPD is a chronic and progressive disease in terms of a subtle decrease of lung function parameters and seasonal aspects in terms of allergic compounds might not be as relevant as in asthmatic patients. On the other hand, exacerbations may also cause an only limited worsening of lung function parameters with a clinical improvement after some days (and a reduction in drug therapy). Therefore, severity assessment will be performed on a regular base as for asthma and a 3 months period seems to appropriate.

6.7.1.3 Definition of treatment steps for patients with COPD and Asthma

Regarding both guidelines (GINA (1) and GOLD (2)), relevant differences have to be mentioned. In asthma patients, ICS is given as first controller medication and LABA usage without ICS is not recommended (at least after the SMART trial (10)) due to the inflammatory etiology of asthma. In contrast, LABA usage without ICS is recommended in COPD patients and ICS is given only to patients suffering from severe COPD. Therefore the following simplified treatment steps can only be used as are very rough assessment of disease severity in patients suffering from these two diseases.

Treatment step 1*: reliever medication only (compounds see above)

Treatment step 2: reliever medication plus addition of long-acting bronchodilative agents (LAMA and /or LABA) and/or inhaled corticosteroids

Treatment step 3: addition of systemic corticosteroids

*Whereas inhaled reliever medication is stated as preferred treatment, other reliever drug (classes) e.g. oral SAMA, SABA and theophylline or nebulized reliever treatments are typically used in patients with a more severe COPD/asthma who have issues with inhaler technique perhaps due to comorbidities and functional status. Due to the increased systemic drug exposure, treatment step 1 will be split in two groups (inhaled, oral reliever medication) which will also be considered for the other treatment steps in the sensitivity analysis.

As for asthma and COPD, severity assessment will be performed on a 3 months period.

6.7.1.4 Consideration of different systemic reliever exposures regarding treatment steps

Whereas inhaled reliever medication is stated as preferred treatment, other reliever drugs (classes) e.g. oral SAMA, SABA and theophylline are typically used for patients with a more severe disease. Due to the increased systemic drug exposure, treatment step 1 will be split in two treatment steps in the sensitivity analyses:

- (1a) Reliever medication only: inhaled SABA or inhaled SAMA
- (1b) Reliever medication only: oral SABA or oral SAMA or short-acting theophylline or patient with both - inhaled and oral - SABA/SAMA combinations

This stratification will also be considered in all treatment steps.

Change to:

~~Since an influence on MI risk cannot be excluded, the severity of asthma and COPD has to be included in our analysis as a confounding factor (59). Unfortunately, severity of both diseases is only rarely depicted in ICD-coding in general. Furthermore, clinically relevant parameters describing disease severity (e.g. lung function parameters, number of hospitalisations) are not documented in most databases. Treatment steps could be useful for defining disease severity but since LABA is used in most treatment steps (particular in asthma~~

patients), methodological aspects limit the results to a huge extent. Therefore, treatment steps will be used in the sensitivity analysis only (8).

Regarding the numerous drugs used in COPD and asthma and possible drug combinations, treatment steps can only be judged as a very rough surrogate for disease severity. Additionally, drugs not primarily recommended in the guidelines (and depicted in the treatment steps) will also be used (e.g. theophylline in COPD patients). Therefore we included the group “combinations not listed” in our treatment steps. Furthermore, prescription behaviour might have changed within the last years affecting the study population (e.g. LABA usage without ICS has been re-assessed after the SMART trial (10)). Therefore, we did change the current asthma treatment step 3 consisting of a fixed combination of LABA/ICS in “LABA or LABA/low dose ICS”. In summary, there are several severe limitations regarding the usage of treatment steps for assessing disease severity.

Furthermore, focussing on drugs only and non considering of clinical issues can only give a limited impression of severity as stated also in the GINA guidelines: “It is important to recognize, however, that asthma severity involves both the severity of the underlying disease and its responsiveness to treatment. Thus, asthma could present with severe symptoms and airflow obstruction, but become completely controlled with low dose treatment”. Regarding the relevance of clinical endpoints we want to use clinical parameters (if available) to refine disease severity assessment in the sensitivity analysis for asthma and COPD patients separately. For the other patients included in our study (COPD & asthma) relevant clinical issues (e.g. hospitalisation) will also be defined.

Since severity will change over time, we will (re)assess severity regarding 3 months intervals. Additionally, treatment steps according to the guidelines can be used only in patients with a diagnosis of asthma or COPD. For patients suffering from both diseases (our 3rd cohort) simplified treatment steps will be used for defining severity.

6.7.1.1 Definition of treatment steps for asthma:

According to recent guidelines published by the Global Initiative for Asthma ([GINA] see figure 4 (1)), drugs can be used for defining treatment steps. In general, asthma (and COPD) drugs can be divided in reliever compounds used as needed medication due to a rapid bronchodilation and in controller medication which is used on a regular base (several long acting, slow acting mechanisms, e.g. anti inflammation). In treatment step 1, reliever medication only is given to the patient but in treatment step 2, controller medication is started (and reliever medication is added on an “as needed base” by the patient).

		Treatment Steps			
		Step 1	Step 2	Step 3	Step 4
Asthma education Environmental control					
As needed rapid-acting β_2 -agonist		As needed rapid-acting β_2 -agonist			
		Select one	Select one	Add one or more	Add one or both
Controller options	Low-dose inhaled ICS [†]	Low-dose ICS plus long-acting β_2 -agonist	Medium- or high-dose ICS plus long-acting β_2 -agonist	Oral glucocorticosteroid (lowest dose)	
	Leukotriene modifier [‡]	Medium- or high-dose ICS	Leukotriene modifier	Anti-IgE treatment	
		Low-dose ICS plus leukotriene modifier	Sustained release theophylline		
		Low-dose ICS plus sustained release theophylline			

[†]Inhaled glucocorticosteroids. [‡]Receptor antagonist or synthesis inhibitors.

Figure 4: Asthma treatment according to the GINA guidelines (preferred controller options are shown in shadowed boxes; figure taken from Hoshino et al. (60). Please note: Whereas “rapid-acting” B2A are stated as “as-needed” medication the GINA guidelines (1), “short-acting” B2A are stated as “as-needed” medication in the British Guideline (4) (see figure 5).

Whereas in some guidelines (e.g. GINA (1)) RABA (rapid acting B2A) are stated as reliever medication in other guidelines (e.g. British guideline on the Management of Asthma (4), see figure 5) SABA are recommended.

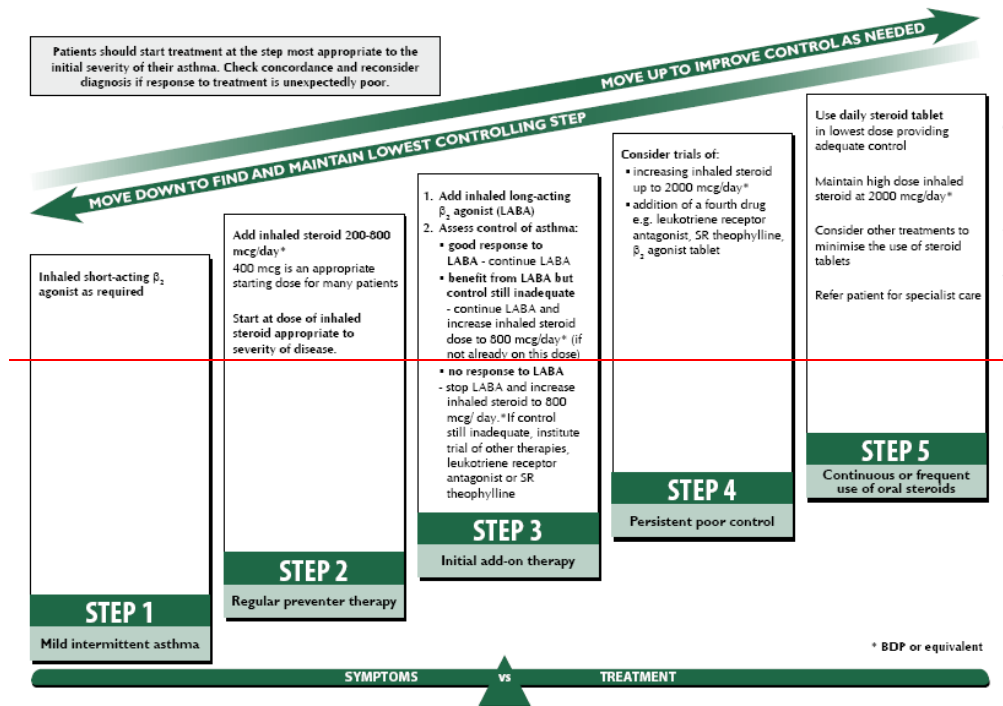


Figure 5: Asthma treatment according to the British Guideline on the Management of Asthma (May 2008, revised May 2011(4)). Please note: Whereas “short-acting” B2A are stated as “as-needed” medication in the British Guideline, “rapid-acting” B2A are stated as “as-needed” medication the GINA guidelines (1) (see figure 4).

As discussed above, formoterol is a rapid-acting LABA (and a member of the RABA class) and might be used in treatment step 1 as reliever medication (see e.g. GINA (1)). On the other hand, formoterol should be used – as already discussed for LABAs and according to the guidelines – in combination with ICS only. Focussing on the effect duration (and the combined usage of LABA and ICS which is widely stated as treatment step 3), we decided to use the conventional classification system (SABA and LABA) as stated e.g. in the recently published British guideline on the Management of Asthma (4) (see figure 5). Thus, we want to reduce confusion regarding formoterol and all patients using this compound will be included in our analysis as at least treatment step 3.

Treatment step 1*: Reliever medication only: SABA, SAMA, short-acting theophylline

Treatment step 2: Low-dose ICS or leukotriene modifier (plus reliever medication)

Treatment step 3: LABA or LABA/low-dose ICS or medium-/high-dose ICS or low-dose ICS plus leukotriene modifier or low-dose ICS plus sustained-release theophylline (plus reliever medication)

Treatment step 4: Treatment step 3 plus one or more of the following: LABA and medium- or high-dose ICS and/or leukotriene modifier and/or sustained-release theophylline

Treatment step 5***: Addition of oral glucocorticosteroid to Step 4

Treatment step 6***: Addition of omalizumab to step 5

Treatment step 7: combinations not listed above

*Since inhaled SABA is the preferred drug class within the reliever medication group and other reliever drug (classes) e.g. oral SABA and theophylline are typically used in patients with a more severe asthma due to the increased systemic drug exposure, treatment step 1 will be split in two groups (inhaled, oral reliever medication) which will also be considered for the other treatment steps in the sensitivity analysis.

**Since omalizumab should only be used as a “last” option, step 5 according to GINA has been divided in two steps (chronic oral corticosteroids [duration of prescription at least 30 days] and omalizumab).

As stated above, time course of severity changes has to be taken into account. Since seasonal influences are well-known particularly for extrinsic (allergic) asthma, a higher asthma severity during spring and summer may occur but in autumn / winter, asthma medication might be reduced. Due to these changes, severity assessment of asthma patients will be performed on a 3-months interval (quarter).

6.7.1.2 Definition of treatment steps for COPD

Using a current guideline of the Global Initiative for Chronic Obstructive Lung Disease (2) (see figure 6), drug therapy can also be used for a rough estimation of the severity of COPD. As in asthma patients treatment of COPD is started using reliever medication “as needed” and by reaching treatment step 2, maintenance medication is given on a regular base (and reliever medication as needed).

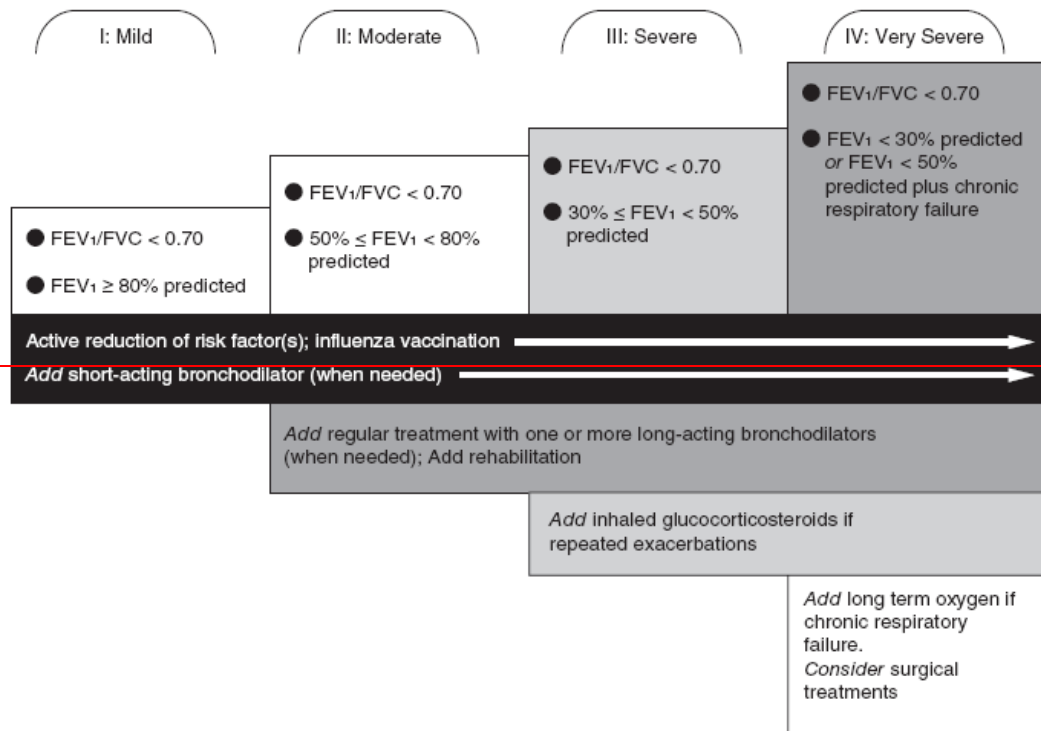


Figure 6: COPD treatment according to GOLD (figure taken from Hoshino et al. (60))

Treatment step 1*: Reliever medication only: SABA, SAMA, short-acting theophylline

Treatment step 2: LAMA or LABA (plus reliever medication)

Treatment step 3: Treatment step 2 plus inhaled corticosteroids

Treatment step 4: Addition of systemic corticosteroids (usually just for acute exacerbations)

Treatment step 5: combinations not listed above (e.g. slow-release theophylline)

*Whereas inhaled reliever medication is stated as preferred treatment, other reliever drug (classes) e.g. oral SAMA, SABA and theophylline are typically used in patients with a more severe COPD. Due to the increased systemic drug exposure, treatment step 1 will be split in two groups (inhaled, oral reliever medication) which will also be considered for the other treatment steps in the sensitivity analysis.

Since non-drug related issues are documented in some databases only, we will use oxygen therapy and surgical interventions defining very severe COPD in the sensitivity analysis only.

As for asthma, time change of severity steps has to be taken into account. From a pathophysiological perspective, COPD is a chronic and progressive disease in terms of a subtle decrease of lung function parameters and seasonal aspects in terms of allergic compounds might not be as relevant as in asthmatic patients. On the other hand, exacerbations may also cause an only limited worsening of lung function parameters with a clinical improvement after some days (and a reduction in drug therapy). Therefore, severity assessment will be performed on a regular base as for asthma and a 3 months period seems to appropriate.

6.7.1.3 Definition of treatment steps for patients with COPD and Asthma

Regarding both guidelines (GINA (1) and GOLD (2)), relevant differences have to be mentioned. In asthma patients, ICS is given as first controller medication and LABA usage without ICS is not recommended (at least

after the SMART trial (10)) due to the inflammatory etiology of asthma. In contrast, LABA usage without ICS is recommended in COPD patients and ICS is given only to patients suffering from severe COPD. Therefore the following simplified treatment steps can only be used as a very rough assessment of disease severity in patients suffering from these two diseases.

Treatment step 1*: reliever medication only (compounds see above)

Treatment step 2: reliever medication plus addition of long acting bronchodilative agents (LAMA and/or LABA) and/or inhaled corticosteroids

Treatment step 3: addition of systemic corticosteroids

~~*Whereas inhaled reliever medication is stated as preferred treatment, other reliever drug (classes) e.g. oral SAMA, SABA and theophylline or nebulized reliever treatments are typically used in patients with a more severe COPD/asthma who have issues with inhaler technique perhaps due to comorbidities and functional status. Due to the increased systemic drug exposure, treatment step 1 will be split in two groups (inhaled, oral reliever medication) which will also be considered for the other treatment steps in the sensitivity analysis.~~

~~As for asthma and COPD, severity assessment will be performed on a 3 months period.~~

~~6.7.1.4 Consideration of different systemic reliever exposures regarding treatment steps~~

~~Whereas inhaled reliever medication is stated as preferred treatment, other reliever drugs (classes) e.g. oral SAMA, SABA and theophylline are typically used for patients with a more severe disease. Due to the increased systemic drug exposure, treatment step 1 will be split in two treatment steps in the sensitivity analyses:~~

~~–(1a) Reliever medication only: inhaled SABA or inhaled SAMA~~

~~–(1b) Reliever medication only: oral SABA or oral SAMA or short acting theophylline or patient with both inhaled and oral SABA/SAMA combinations~~

~~This stratification will also be considered in all treatment steps.~~

6.8 Additional surrogates for defining disease severity

Change from:

Because the definition of severity with drugs as surrogate parameter is imprecise imprecise and treatment guidelines are not useful due to the linkage to the exposure (LABA is the exposure and is an important factor for the definition of the treatment steps), clinical and other endpoints will be used for defining severity for the 3 patient groups as follows (if available in the respective dataset):

Change to:

6.8 Additional surrogates for defining disease severity (high priority)

Because the definition of severity with drugs as surrogate parameter is imprecise imprecise and treatment guidelines are not useful due to the linkage to the exposure (LABA is the exposure and is an important factor for the definition of the treatment steps), clinical and other endpoints will be used for defining severity for the 3 patient groups as follows (if available in the respective dataset):

6.8 Additional surrogates for defining disease severity

Change from:

6.8.1 For Asthma:

i.) Use of health care providers

- Number of asthma-related GP contacts
- Number of encounters with pulmonologist or allergist
- Number of encounters with cardiologist
- Number of asthma-related emergency contacts
- Number of asthma-related hospital admissions (asthma-related admission diagnosis or any other respiratory related hospitalisation)
- Any pulmonary function testing (regardless of available data on results)

ii.) Lung function parameters

- FEV1 [Forced Expiratory Volume in 1 second, % predicted]
- Peak flow

It must be considered, that by the recently published GINA guidelines (1), a huge change has been made. In comparison to previous guidelines, the discrimination of lower levels of % predicted FEV1 has been stopped and instead, a flexible “level of control” has been introduced for guiding physicians in asthma treatment. Therefore, the relevance of % predicted FEV1 has to be discussed.

iii.) Controller total asthma medication ratio (61)

iv.) # of SABA prescriptions/dispensing in prior 12 months

v.) # of short courses of oral corticosteroids (OCS) or antibiotic prescriptions within two weeks of GP or specialist encounter for respiratory-related diagnosis (ICD class J) as proxy for asthma exacerbation

6.8.2 For COPD:

i.) Use of health care providers

- Number of COPD-related GP contacts
- Number of encounters with pulmonologist or allergist
- Number of encounters with cardiologist
- Number of COPD-related emergency contacts
- Number of COPD-related hospital admissions (COPD-related admission diagnosis or any other respiratory-related hospitalisation)
- Any pulmonary function testing (regardless of available data on results)

ii.) Lung function parameters

- $FEV1$ [Forced Expiratory Volume in 1 second] / FVC [Forced Vital Capacity] < 0.70
- $FEV1$ [Forced Expiratory Volume in 1 second] % predicted Use
-

iii.) # of SABA/SAMA prescriptions/dispensing in prior 12 months

iv.) # of short courses of OCS or antibiotic prescriptions within two week of GP or specialist visit for respiratory-related diagnoses as proxy for moderate COPD exacerbation.

v.) Use (prescription) of oxygen / oxygen inhalation devices

vi.) COPD-associated lung volume reduction surgery (due to emphysema)

6.8.3 For asthma and COPD patients

i.) Use of health care providers

- Number of COPD-/asthma-related GP contacts
- Number of encounters with pulmonologist or allergist
- Number of encounters with cardiologist
- Number of COPD-/asthma-related emergency contacts
- Number of COPD-/asthma-related hospital admissions (COPD-/asthma-related admission diagnosis or any other respiratory-related hospitalisation)

ii.) Lung function parameters

- FEV1 [Forced Expiratory Volume in 1 second] % predicted
- Peak flow
- FEV1 [Forced Expiratory Volume in 1 second] / FVC [Forced Vital Capacity]

iii.) # of SABA/SAMA prescriptions/dispensing in prior 12 months

iv.) # of short courses of OCS or antibiotic prescriptions within two week of GP or specialist visit for respiratory-related diagnoses as proxy for moderate COPD / asthma exacerbation.

v.) Use (prescription) of oxygen / oxygen inhalation devices

iv.) COPD-associated lung volume reduction surgery (due to emphysema)

Change to:

6.8.1 For Asthma:

i.) Use of health care providers

- Number of asthma-related GP contacts
- Number of encounters with pulmonologist or allergist
- Number of encounters with cardiologist
- Number of asthma-related emergency contacts
- Number of asthma-related hospital admissions (asthma-related admission diagnosis or any other respiratory related hospitalisation)
- Any pulmonary function testing (regardless of available data on results)

ii.) Lung function parameters

- FEV1 [Forced Expiratory Volume in 1 second, % predicted]
- Peak flow

It must be considered, that by the recently published GINA guidelines (1), a huge change has been made. In comparison to previous guidelines, the discrimination of lower levels of % predicted FEV1 has been stopped and instead, a flexible "level of control" has been introduced for guiding physicians in asthma treatment. Therefore, the relevance of % predicted FEV1 has to be discussed.

iii.) Controller total asthma medication ratio (61)

iv.) # of SABA prescriptions/dispensing in prior 12 months / **increase in SABA prescriptions in prior 12 month (absolute numbers and/or per cent rise)**

v.) # of short courses of oral corticosteroids (OCS) or antibiotic prescriptions within two weeks of GP or specialist encounter for respiratory-related diagnosis (ICD class J) as proxy for asthma exacerbation

6.8.2 For COPD:

i.) Use of health care providers

- Number of COPD-related GP contacts
- Number of encounters with pulmonologist or allergist
- Number of encounters with cardiologist
- Number of COPD-related emergency contacts
- Number of COPD-related hospital admissions (COPD-related admission diagnosis or any other respiratory-related hospitalisation)
- Any pulmonary function testing (regardless of available data on results)

ii.) Lung function parameters

- FEV1 [Forced Expiratory Volume in 1 second] / FVC [Forced Vital Capacity] < 0.70
- FEV1 [Forced Expiratory Volume in 1 second] % predicted Use
-

iii.) # of SABA/SAMA prescriptions/dispensing in prior 12 months / **increase in SABA prescriptions in prior 12 month (absolute numbers and/or per cent rise)**

iv.) # of short courses of OCS or antibiotic prescriptions within two week of GP or specialist visit for respiratory-related diagnoses as proxy for moderate COPD exacerbation.

v.) Use (prescription) of oxygen / oxygen inhalation devices

vi.) COPD-associated lung volume reduction surgery (due to emphysema)

6.8.3 For asthma and COPD patients

i.) Use of health care providers

- Number of COPD-/asthma-related GP contacts
- Number of encounters with pulmonologist or allergist
- Number of encounters with cardiologist

- Number of COPD-/asthma-related emergency contacts
- Number of COPD-/asthma-related hospital admissions (COPD-/asthma-related admission diagnosis or any other respiratory-related hospitalisation)

ii.) Lung function parameters

- FEV1 [Forced Expiratory Volume in 1 second] % predicted
- Peak flow
- FEV1 [Forced Expiratory Volume in 1 second] / FVC [Forced Vital Capacity]

iii.) # of SABA/SAMA prescriptions/dispensing in prior 12 months / **increase in SABA prescriptions in prior 12 month (absolute numbers and/or per cent rise)**

iv.) # of short courses of OCS or antibiotic prescriptions within two week of GP or specialist visit for respiratory-related diagnoses as proxy for moderate COPD / asthma exacerbation.

v.) Use (prescription) of oxygen / oxygen inhalation devices

iv.) COPD-associated lung volume reduction surgery (due to emphysema)

6.9 Other factors influencing AMI risk

Change from:

Some co-medication and co-morbidities might have only a minor influence on the AMI risk. These factors will also be considered in the sensitivity analysis.

Change to:

6.9 Other **co-medication factors influencing AMI risk**

Some co-medication **and co-morbidities** might have only a minor influence on the AMI risk. These factors will also be considered in the sensitivity analysis.

6.10 Socioeconomic status

Change from:

In databases where data is available for socioeconomic status, it will be included as an additional confounder in the sensitivity analysis

Change to:

~~In databases where data is available for socioeconomic status, it will be included as an additional confounder in the sensitivity analysis~~

