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Comparative safety of asthma treatment regimens during pregnancy and related methodological aspects

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Comparative safety of asthma treatment regimens during pregnancy and related methodological aspects

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Résumé

L'asthme est l'une des maladies chroniques les plus fréquentes durant la grossesse, affectant environ 4% à 12% des femmes enceintes et ayant une prévalence qui a augmenté au cours des dernières décennies. Plusieurs études ont identifié l'asthme comme un facteur de risque pour plusieurs enjeux de santé défavorables chez le fœtus et la mère. Les lignes directrices de traitement recommandent l'utilisation de médicaments antiasthmatiques pendant la grossesse afin de contrôler l'asthme et d'éviter les problèmes de santé maternels et fœtaux. L'évaluation de la littérature sur l'utilisation maternelle de médicaments antiasthmatiques et le risque de malformations congénitales majeures a relevé plusieurs études sur l'innocuité des bêta$_2$-agonistes inhalés à courte durée d'action (BACA) et des corticostéroïdes inhalés (CSI) pendant la grossesse, mais peu de données sur les bêta$_2$-agonistes à longue durée d'action (BALA) ainsi que sur les thérapies combinées (BALA-CSI). Un programme de recherche en trois volets a été développé pour combler ces lacunes. Dans le premier volet, nous avons entrepris une revue systématique de la littérature sur l'impact de l'utilisation de BACA et de BALA pendant la grossesse sur le risque de différents problèmes périnataux. Vingt et une études originales ont été identifiées. Quatre études ont rapporté une augmentation significative du risque de malformations congénitales avec BACA, une étude a rapporté une augmentation significative du risque de malformations congénitales avec BALA et quatre études ont rapporté un risque significatif accru de malformations congénitales avec bêta$_2$-agonistes (BACA et/ou BALA). Toutefois, aucun risque majeur n'a été trouvé pour les autres complications périnatales. Fait important, la plupart des études récupérées ont subi plusieurs limitations méthodologiques, y compris l'utilisation des femmes non-asthmatiques comme groupe de référence et la faible puissance statistique. De plus, les résultats qui en découlent doivent être interprétés avec prudence. Dans le deuxième volet, nous avons utilisé la base de données *Québec Asthma and Pregnancy Database* qui comprend toutes les grossesses de femmes asthmatiques et un échantillon aléatoire de femmes non-asthmatiques ayant accouchées entre 1990 et 2010 pour effectuer deux études. La première était une étude comparant la prévalence des malformations congénitales majeures entre les femmes enceintes asthmatiques traitées avec une combinaison de BALA-CSI et celles traitées avec une dose plus élevée de CSI en

monothérapie. Dans une sous-cohorte, il y'avait 643 femmes qui utilisaient un BALA plus CSI à dose faible et 305 qui ont utilisé une dose moyenne de CSI ; l'autre sous-cohorte comprenait 198 utilisatrices de BALA plus CSI à dose moyenne et 156 utilisatrices de CSI à dose élevée. La prévalence de malformations majeures a été 6,9% et 7,2%, respectivement. Le risque de malformations congénitales majeures était similaire entre ces deux groupes de femmes avec un odds ratio ajusté (OR) de 1,1 (IC 95%: 0,6-1,9) pour les femmes souffrant d'asthme modéré et un OR ajusté de 1,2 (IC 95%: 0,5-2,7) pour les femmes souffrant d'asthme sévère. La seconde était une étude méthodologique visant à étudier l'impact de six différentes définitions opérationnelles de malformations congénitales qui varient selon la source des données et la méthode de classification sur l'estimation de la prévalence des malformations et de l'association entre l'asthme maternel et les malformations majeures. Sur 467,946 grossesses, 12,3% étaient de femmes enceintes souffrant d'asthme actif. Nous avons démontré que la source des données et la méthode de classification ont eu un impact considérable sur la prévalence des malformations congénitales majeures (augmentation entre 10,0% et 50,4%), alors qu'elles ont eu peu d'influence sur l'association entre l'asthme maternel et les malformations congénitales. Dans le troisième volet du programme de recherche, nous avons développé une procédure systématique pour la classification des médicaments utilisés au cours du premier trimestre de grossesse en agents tératogènes et potentiellement tératogènes dans un contexte de recherche. Nous avons développé une procédure systématique qui s'actualise facilement, avec des composantes objectives dans la plupart de ses processus. Nous avons établi une liste comprenant 91 médicaments tératogènes, et une autre liste comprenant 81 médicaments potentiellement tératogènes. Les résultats présentés dans cette thèse ont fourni des données importantes sur l'innocuité des traitements de l'asthme pendant la grossesse, aidant les cliniciens et les femmes enceintes à choisir un traitement pharmacologique sécuritaire pour maintenir l'asthme sous contrôle. De plus, les données présentées dans cette thèse sur la minimisation du biais d'indication, les définitions opérationnelles de malformations congénitales et l'identification des médicaments tératogènes pourront aisément être utilisées par les chercheurs en pharmacoépidémiologie, en tératologie et en épidémiologie périnatale.

Mots-clés : asthme, grossesse, bêta$_2$-agonistes, corticostéroïdes, malformations congénitales, tératogènes.

Abstract

Asthma is one of the most prevalent chronic diseases during pregnancy, affecting about 4% to 12% of pregnant women and shows an increasing prevalence over time. In the past decades, several studies have identified asthma as a risk factor for several poor fetal and maternal outcomes. A consensus exists on favoring the use of asthma medications during pregnancy to maintain asthma under control to prevent adverse maternal and fetal outcomes. An assessment of the published literature on maternal asthma medications and the risk of major congenital malformations revealed more data on the safety of short-acting beta$_2$-agonists (SABA) and inhaled corticosteroids (ICS) during pregnancy compared to long-acting beta$_2$-agonists (LABA), as well as a paucity of data on the fetal safety of combination therapies (e.g. LABA-ICS). A three-part research program was developed to fill this knowledge gap and answer other intriguing questions we faced, adding necessary evidence in this field. In the first part, we summarized the published evidence on the impact of maternal use of SABA and LABA during pregnancy and different perinatal outcomes in a comprehensive systematic review. Twenty-one original studies were identified. Four studies reported a significant increased risk of congenital malformations with SABA, one study reported a significant increased risk of congenital malformations with LABA and four studies reported a significant increased risk of congenital malformations with beta$_2$-agonists (SABA and/or LABA). However, no major increased risk was found for the other perinatal outcomes. Importantly, most of the retrieved studies suffered several methodologic limitations, including using non-asthmatic women as the reference group and low statistical power. Moreover, the non-significant results reported should be interpreted with caution. In the second part, we used the *Quebec Asthma and Pregnancy Database* – which includes all pregnancies in asthmatic women and a random sample in nonasthmatic women between 1990 and 2010 – to conduct two studies. The first was a comparative safety study examining the prevalence of major congenital malformations in pregnant

asthmatic women treated with a combination of LABA-ICS compared to those treated with a higher dose of ICS monotherapy. In one subcohort there were 643 women who used a LABA plus low-dose ICS and 305 women who used a medium-dose ICS; the other subcohort included 198 users of a LABA plus a medium dose ICS and 156 users of a high-dose ICS. The prevalence of major malformations was 6.9% and 7.2%, respectively. The risk of major malformations did not differ when a combination therapy was used among both moderate and severe asthmatic women (aOR: 1.1; 95% CI: 0.6–1.9 and aOR: 1.2; 95% CI: 0.5–2.7 respectively). The second was a methodological study aiming to compare the prevalence of major malformations using six different case ascertainment definitions that vary by the source of data and the classification method, as well as to evaluate the impact of these definitions on the association between maternal asthma and major malformations. From the 467,946 pregnancies, 12.3% were with active asthma. We demonstrated that the source of data and the classification method had a considerable impact on the prevalence of major malformations (increases between 10.0% and 50.4%), but only a small influence on the measure of association. In the third part of the research program, we aimed at constructing a systematic procedure for the classification of proven and potential teratogenic medications during the first trimester of pregnancy to be used for research. We structured a procedure that is both systematic and updatable, with objective components in most of its processes. We identified a substantial list of teratogenic medications, including 91 medications, and an extensive list of potentially teratogenic medications, including 81 medications. The results presented in the current thesis provided essential evidence on the safety of asthma treatments during pregnancy, helping clinicians and mothers to choose the optimal therapeutic regimen to keep asthma under control. The added knowledge on indication bias minimization, congenital malformations ascertainment and teratogenic medications are directly transferable to researchers in pharmacoepidemiology, teratology and other related research fields.

Keywords: asthma, pregnancy, beta$_2$-agonists, corticosteroids, congenital malformations, teratogens.

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Abbreviations

ACASS: Alberta Congenital Anomalies Surveillance System

ACQ : Asthma Control Questionnaire

ACT : Asthma Control Test

aOR: Adjusted Odds Ratio

ATAQ: Asthma Therapy Assessment Questionnaire

BMI: body mass index

CCASS: Canadian Congenital Anomalies Surveillance System

CI: Confidence Interval

CIHI: Canadian Institute for Health Information

cOR: Crude Odds Ratio

cRR: Crude Relative Risk

DAD: discharge abstract data

DAG: Directed acyclic graph

DIN: Drug Identification Number

ED: Emergency Department

FDA: Food and Drug Administration

FEV_1: Forced Expiratory Volume in 1 second.

FRC: functional residual capacity

GEE: Generalized Estimating Equations

GINA: Global Initiative for Asthma

GPRD: General Practice Research Database

HPA: hypothalamic-pituitary-adrenal

ICD: International Classification of Disease

ICS: Inhaled Corticosteroids

IFN-γ : interferon γ

IL- 4 : interleukin-4

INCS: Intranasal Corticosteroids

IUGR : Intrauterine Growth Retardation

LABA: Long Acting $Beta_2$-Agonists

LBW: low birth weight

LTRAs: Leukotriene Receptor Antagonists

MED-ECHO : Maintenance et exploitation des données pour l'étude de la clientèle hospitalière

NAEPP: National Asthma Education and Prevention Program

NBDPS: National Birth Defects Prevention Study

NK: natural killer cells

NOS : Newcastle-Ottawa Scale

NTD: neural tube defects

OCS: Oral Corticosteroids

OR: Odds Ratio

OTC: over the counter

PNV: predictive negative value

pOR: Prevalence Odds Ratio

PPV: predictive positive value

RAMQ : Régie de l'Assurance Maladie du Québec

SABA: Short Acting $Beta_2$-Agonists

SGA: small for gestational age

TCMC: Two-step Congenital Malformation Classification

TERIS: Teratogen Information System

Th2: T-helper cell type 2

THIN: The Health Improvement Network primary care database

Tregs: regulatory T cells

US: United States

V_E: Minute ventilation

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CHAPTER 1: INTRODUCTION

Introduction

Asthma is one of the most prevalent chronic diseases during pregnancy.[1] The disease affects about 4% to 12% of pregnant women and shows an increasing prevalence over time.[2-6] In the past decades, several studies have identified asthma as a risk factor for several poor fetal and maternal outcomes. Numerous studies have shown associations between suboptimal control of asthma and more severe asthma during pregnancy and increased maternal and fetal risks.[2,5,7-9] In contrast, better-controlled asthma and mild-to-moderate actively managed asthma are associated with decreased risks.[10,11] A consensus has been formed through the years on favoring the use of asthma medications during pregnancy to maintain asthma under control to prevent adverse maternal and fetal outcomes.[5,12]

The ultimate goal of asthma therapy in pregnancy is maintaining adequate oxygenation for the fetus and preventing maternal hypoxic episodes.[13] Asthma medications are categorized into two classes; 1) quick relief medications (e.g. short-acting $beta_2$-agonists [SABA]), and 2) long-term controller medications (e.g. inhaled corticosteroids [ICS] and long-acting $beta_2$-agonists [LABA]). SABA have been widely used for years for the quick relief of asthma symptoms during pregnancy, while ICS are considered the mainstay of controller therapy during pregnancy.[5,14] More data on the safety of SABA and ICS during pregnancy are available in the literature compared to LABA due to their precedence in the markets.[15] LABA are used for patients with moderate and severe persistent asthma not fully controlled with inhaled corticosteroids alone.[15]

According to recent reports from the Public Health Agency of Canada, major congenital malformations are present in approximately 3%–5% of newborns and 8%–10% of stillbirths in Canada.[16] Major congenital malformations are considered among the leading causes of infant, fetal, and post neonatal mortality in North America and Europe.[16-20] Ten studies investigated the risk of congenital malformations associated with the use of SABA and LABA separately.[21-30] Among these studies, five used a control group of asthmatic women[23,24,27,30,31] with two reporting a significant increased risk of congenital malformations,[23,30] the first with Fenoterol use (SABA)[23] and the second with LABA use.[30]

The association between maternal ICS use and the risk of congenital malformations was examined in twenty-three studies.[21,22,28,29,32-50] ICS users were compared with women with asthma who did not use any ICS during pregnancy in six studies [39,41,43,44,49,51]. Moreover, one study examined fluticasone against other ICS [33] and one study examined high ICS dose compared to lower ICS dose.[34] A significant increased risk of all malformations was found in one study when high daily doses of ICS (>1000 mg/d equivalent beclomethasone dipropionate) were compared to lower daily doses of ICS (> 0-1000 mg/d) (adjusted odds ratio [OR], 1.66; 95%CI 1.02, 2.68).[34]

Current recommendations for the addition of LABA to ICS - for persistent asthmatic pregnant women - are based on the established evidence of better asthma control with a combination therapy compared to ICS monotherapy outside of pregnancy.[14,52] However, the evidence on the fetal safety of the combination therapy is scarce. Women with persistent asthma are encouraged to continue taking their asthma medications if pregnancy occurs and should be managed optimally with the right treatment regimen that reduces the adverse asthma symptoms and exacerbations. One of the important clinical decisions that the physician has to make is to whether prescribe LABA in addition to the current dose of ICS or increase the ICS dose, if asthma cannot be controlled with low dose ICS.

Combination treatment regimens (LABA plus ICS) were examined in three recent studies [28,33,35], two of which used asthmatics and non-asthmatics as a reference group.[28,35]. The third study examined fluticasone and salmeterol combination users against other ICS monotherapy users and did not find a significant difference.[33] However, some methodologic limitations were present. To the best of our knowledge, we found no study that compared the risk of congenital malformations between women exposed to combination therapy (LABA plus ICS) and women exposed to ICS monotherapy at higher doses during pregnancy.

Since administrative health data are collected for administrative and payment purposes, researchers planning on using these databases should identify the possible threats to their studies validity and apply strategies to tackle these limitations. The use of accurate and valid operational definitions for the outcomes of interest is essential. Regarding

congenital malformations research, discrepancies in the estimated prevalence of major malformations can be easily located in prior reports that used computerized administrative databases, which can be attributed to several factors including the source of data, the diagnostic codes validity, the classification method, and the period of assessment.[53-55] All these factors should be considered in specifying and developing the case ascertainment definitions in pharmacoepidemiologic studies.

Quebec's Medical Claims database - *Régie de l'Assurance-Maladie du Québec* (RAMQ) - and the hospitalisations records - *Maintenance et Exploitation des Données pour l'Étude de la Clientèle Hospitalière* (MED-ECHO) - have been used previously for congenital malformations research.[56-59] The accuracy of congenital malformations diagnoses recorded in RAMQ and MED-ECHO databases was reported in two studies.[60,61] However, no study has investigated the prevalence of congenital malformations diagnoses reported in each database separately. Beside the variation in the prevalence estimates, the case ascertainment definitions might also influence the estimates of the associations between maternal exposures and congenital malformations.

In observational studies of congenital malformations, it is essential to control for the maternal exposure to proven and potential teratogenic medications, as failure to do so can affect the study validity. The increase in the body of knowledge on currently used medications make it difficult to identify a list of teratogenic medications that should be used in research. While strong evidence of teratogenicity exists for some medications (e.g. thalidomide), the evidence is not conclusive for most of the currently used medications. Several databases and references on teratogenic risks are currently available, providing either complete or partial evidence for the teratogenicity of medications.[62-73] However, there are substantial discrepancies between the lists of medications that should be considered teratogenic, and significant imprecision is added when categories are used (e.g., moderate- vs high-risk teratogens).[62-73]. Moreover, the current lists of teratogenic medications used in research are outdated and require constant review of the literature to incorporate the newly generated evidence and recent updates.[65] Therefore, harnessing the full potential of several reliable resources is essential to the creation of a comprehensive overview.

This thesis consists of six chapters and we present it by articles; including one systematic review (published; 2014), one comparative safety study (published; 2015), one methodological study (published; 2016) and one evidence synthesis review (published; 2016). The studies are in a journal manuscript format presented with their figures, tables and references in Chapter 5. The thesis contains separate chapters for the introduction, review of the literature, objectives, methods and discussion.

In the first part of this thesis, we present a systematic review in which we aimed to summarize the existing human data on the impact of the use of inhaled SABA and LABA for the treatment of asthma during pregnancy on several perinatal outcomes, which are major and any congenital malformations, small for gestational age, birth weight, low birth weight, gestational age and preterm delivery.

In the second part of this thesis, we used the *Quebec Asthma and Pregnancy Database*, which includes all pregnancies in asthmatic women and a random sample of pregnancies in nonasthmatic women between January 1, 1990 and March 31, 2010, to conduct two studies. The first was a comparative safety study examining the prevalence of major congenital malformations in pregnant asthmatic women treated with a combination of LABA and ICS compared to those treated with a higher dose of ICS monotherapy. The second study was a methodological study aiming to compare the prevalence of major malformations using different case ascertainment definitions that vary by the source of data and the classification method, and to evaluate the impact of these definitions on the association between maternal asthma and major malformations.

Given the observational nature of the studies included in the current thesis and generally in the field of congenital malformations, the third part of the thesis present our approach to tackling the issue of the discrepancies and inconsistencies of teratogens lists that can be used in perinatal and reproductive research. Using reliable references and resources, we developed a systematic and updatable procedure for the classification of medications into those with sufficient human evidence of teratogenic risk and those with potential teratogenic risk during the first trimester of pregnancy. Finally, we will discuss in

chapter 6 the different strengths and limitations of our research projects and summarize their implications for practice and future research opportunities.

CHAPTER 2: REVIEW OF THE LITERATURE

Review of the literature

2.1 Asthma definition

Asthma is a complex disorder characterized by variable and recurring symptoms, airflow obstruction, bronchial hyperresponsiveness, and an underlying inflammation.[74] Asthma hyperresponsiveness leads to recurrent episodes of wheezing, breathlessness and coughing. Asthma episodes – typically characterized by airflow obstruction – are often reversible, spontaneously or with the use of asthma treatments.[14] The onset of asthma for most patients begins early in life with the pattern of disease persistence determined by early, recognizable risk factors including atopic disease, recurrent wheezing, and a parental history of asthma.[74] For some patients, the development of chronic inflammation may be associated with permanent alterations in the airway structure—referred to as airway remodeling—that are not prevented by or fully responsive to currently available treatments.[75] Therefore, the paradigm of asthma has been expanded over the last 10 years from bronchospasm and airway inflammation to include airway remodeling in some patients.[74,76]

2.2 Asthma prevalence

Asthma is considered one of the most common chronic diseases, affecting as many as 300 million people worldwide.[14,74] Moreover, higher estimates were observed with less conservative criteria for the diagnosis of clinical asthma.[74] The prevalence of asthma have markedly increased over the last 60 years, especially in western countries, where it represents now a considerable burden on the individuals and the healthcare systems.[14,77] The prevalence of asthma in Canada and the United States is among the highest in the world, reaching over 13% for children and about 8% to 12% for adults.[78-81]

2.3 Asthma severity and control

While being complementary notions in the management of asthma, asthma severity and control may overlap in their ways of assessment but each has its distinguished clinical importance.[14,74] The severity of asthma could influence the control over time. Canadian experts have recommended that the dose of inhaled corticosteroids necessary to obtain good control of asthma should be included when evaluating asthma severity level.[52,82]

2.3.1 Asthma severity

Asthma severity is defined as the intrinsic intensity of the disease process.[74] According to GINA 2015, asthma severity is assessed retrospectively from the level of treatment required to control symptoms and exacerbations.[14] It is not a static feature and may change over months or years.[14] Severity is most easily and directly measured in a patient who is not receiving long-term control therapy.[80] Severity can also be measured, once asthma control is achieved, by the step of care required to maintain control.[74] According to United States (US) National Asthma Education and Prevention Program (NAEPP) guidelines for the diagnosis and management of asthma, asthma severity is measured taking into account the level of asthma symptoms, night-time symptoms, use of SABA, pulmonary function and airway limitation, rate of exacerbations, and limitations to normal activities.[74] Using these factors, asthma severity level can be classified into four categories; intermittent, mild persistent, moderate persistent, and severe persistent (see Table 2.3.1 and Table 2.3.2).[74] The stepwise approach for managing asthma is then used to manage asthma in each patient according to his/her severity level (see Figure 2.3.1 and Table 2.3.2).

Table 2.3.1 Classification of asthma severity measured before treatment is started according to US National Asthma Education and Prevention Program guidelines

	Clinical features before treatment[#]		
	Symptoms[¶]	**Night-time symptoms**	**Lung function**
Severe persistent	Continual symptoms Limited physical activity Frequent exacerbations	Frequent	FEV_1 or PEF ≤60% pred PEF variability >30%
Moderate persistent	Daily symptoms Daily use of inhaled SABA Exacerbations affect activity Exacerbations more than twice per week; may last days	More than once per week	FEV_1 or PEF >60 and ≤80% pred PEF variability >30%
Mild persistent	Symptoms more than twice per week but no more than once per day Exacerbations may affect activity	More than twice per month	FEV_1 or PEF ≥80% pred PEF variability 20–30%
Intermittent	Symptoms no more than twice per week Asymptomatic and normal PEF between exacerbations Exacerbations are brief (from a few hours to a few days); intensity may vary	No more than twice per month	FEV_1 or PEF ≥80% pred PEF variability <20%

Asthma severity was classified by clinical characteristics before treatment. FEV_1: forced expiratory volume in one second; PEF: peak expiratory flow; % pred: % predicted; SABA: short-acting β_2-agonist. [#]: the presence of one of the features of severity is enough to place the patient in that category. An individual should be assigned to the most severe grade in which any feature occurs. An individual's classification may change over time. [¶]: Patients at any level can have mild, moderate or severe exacerbations. Some patients with intermittent asthma experience severe and life-threatening exacerbations separated by long periods of normal lung function and no symptoms.

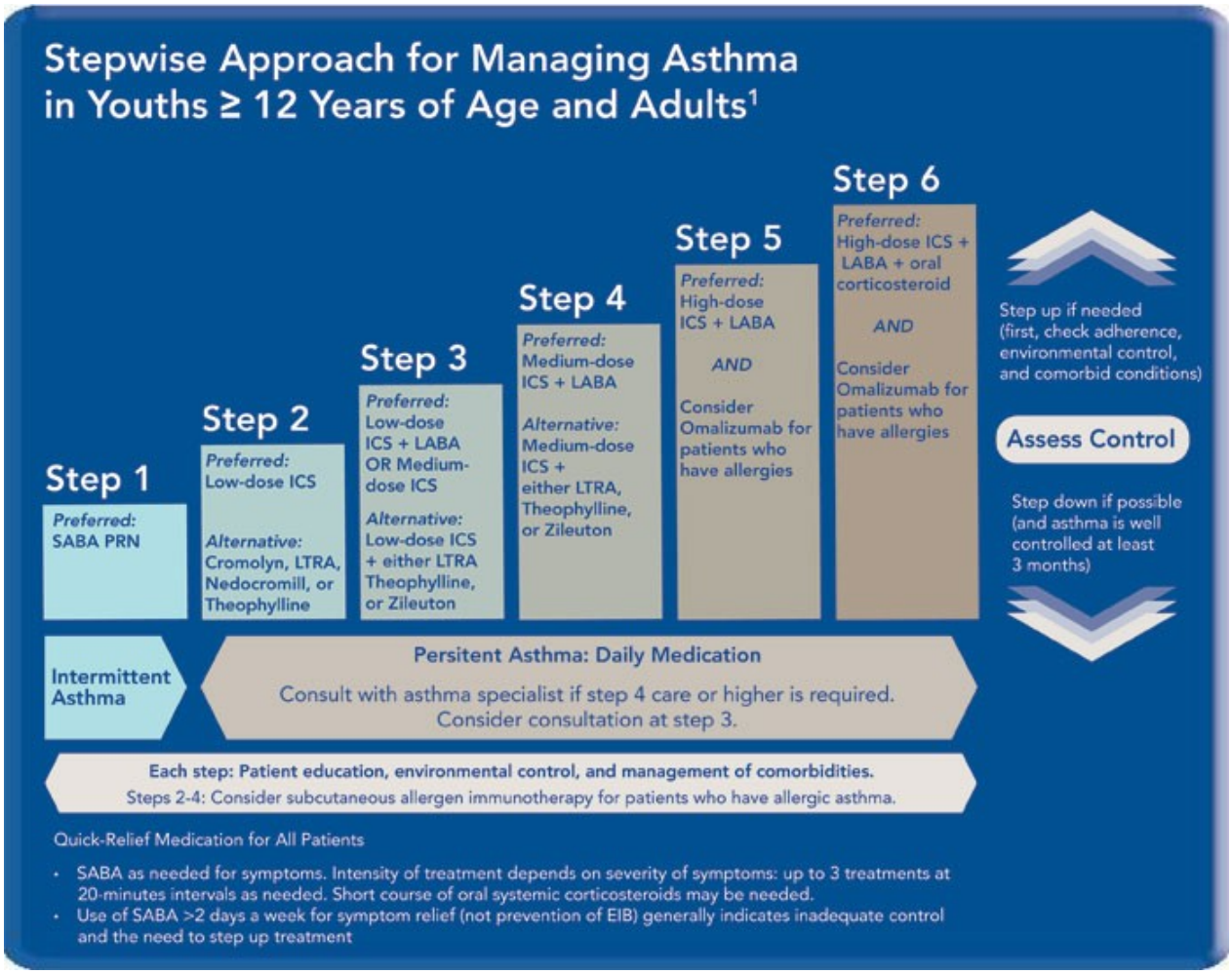
From US National Asthma Education and Prevention Program guidelines for the diagnosis and management of asthma, Expert Panel Report 3, 2007

Table 2.3.2 Classifying severity in patients after asthma becomes well controlled, by lowest level of treatment required to maintain control.

Classification of Asthma Severity			
Intermittent	**Persistent**		
	Mild	Moderate	Severe
Step 1	Step 2	Step 3 or 4	Step 5 or 6

From US National Asthma Education and Prevention Program guidelines for the diagnosis and management of asthma, Expert Panel Report 3, 2007

Figure 2.3.1 Stepwise approach for managing asthma in adults according to the US National Asthma Education and Prevention Program guidelines



Source: US National Asthma Education and Prevention Program guidelines for the diagnosis and management of asthma, Expert Panel Report 3, 2007

2.3.2 Asthma control

According to the US NAEPP guidelines, asthma control is defined as the degree to which the manifestations of asthma are minimized by therapeutic intervention and the goals of therapy are met.[74] Asthma control is assessed through measuring the components of control, including the level of asthma symptoms, night-time awakenings, interference with normal activities, SABA use for quick relief, pulmonary function (FEV_1 or peak flow), exacerbations, the progressive loss of lung function, and the treatment related side-effects.[74] The NAEPP guidelines classifies asthma control into three classes; well controlled, not well controlled and very poorly controlled (see Table 2.3.3).[74]

Table 2.3.3 Classification of asthma control in adults according to US National Asthma Education and Prevention Program guidelines

COMPONENTS OF CONTROL	CLASSIFICATION OF ASTHMA CONTROL		
	WELL CONTROLLED	**NOT WELL CONTROLLED**	**VERY POORLY CONTROLLED**
Impairment			
Symptoms	≤ 2 days/week	> 2 days/week	Throughout the day
Night-time awakenings	≤ 2 times/month	1–3 times/week	≥ 4 times/week
Interference with normal activity	None	Some limitation	Extremely limited
Short-acting beta-2-agonist use for symptom control (not prevention of exercise-induced bronchospasm)	≤ 2 days/week	> 2 days/week	Several times/day
FEV_1 or peak flow	> 80% predicted or personal best	60%–80% predicted or personal best	< 60% predicted or personal best
Validated questionnaires			
ATAQ	0	1–2	3–4
ACQ	≤ 0.75	≥ 1.5	NA
ACT	≥ 20	16–19	≤ 15

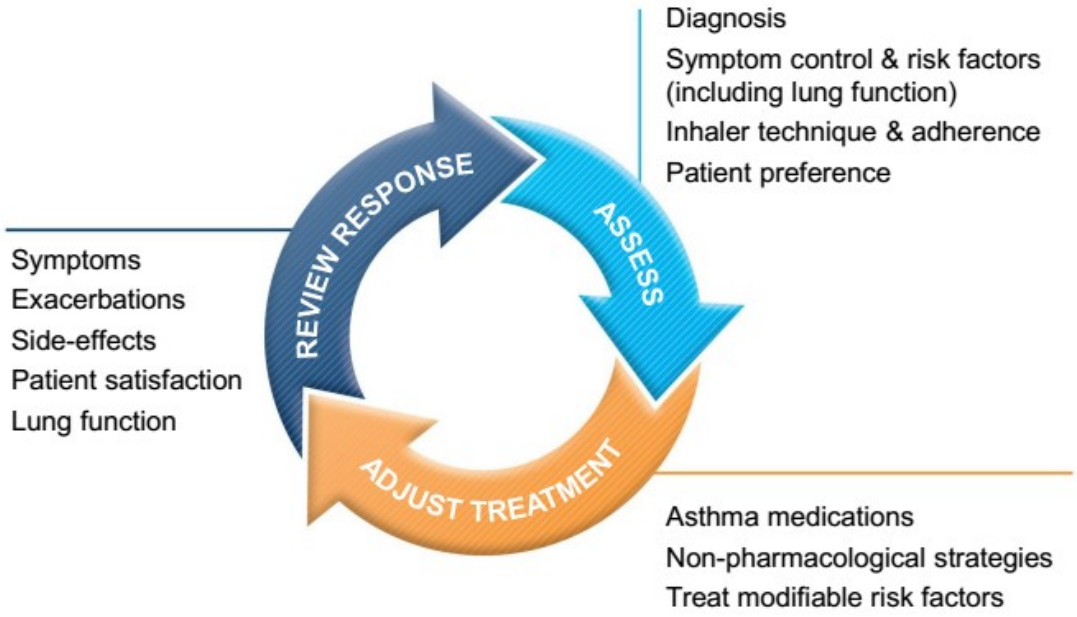
Risk			
Exacerbations requiring oral systemic corticosteroids	0–1/year	2–3/year	> 3/year
	Consider severity and interval since last exacerbation.		
Progressive loss of lung function	Evaluation requires long-term follow-up care.		
Treatment-related adverse effects	Medication side effects can vary in intensity from none to very troublesome and worrisome. The level of intensity does not correlate to specific levels of control but should be considered in the overall assessment of risk.		

ATAQ = Asthma Therapy Assessment Questionnaire, ACQ = Asthma Control Questionnaire, ACT = Asthma Control Test, FEV_1 = forced expiratory volume in 1 second.

From the US National Asthma Education and Prevention Program guidelines for the diagnosis and management of asthma, Expert Panel Report 3, 2007

The GINA guidelines uses similar aspects for measuring asthma control, and classifies it into controlled, partly controlled, and uncontrolled.[14] In control-based asthma management, pharmacological and non-pharmacological treatment is adjusted in a continuous cycle that involves assessment, treatment and review as presented in Fig 2.3.2.[14] Asthma outcomes have been shown to improve after the introduction of practical tools for implementation of control-based management strategies.[14]

Figure 2.3.2 The control-based asthma management cycle (GINA 2015)



Source: *The Global Strategy for Asthma Management and Prevention,* Global Initiative for Asthma (GINA) 2015. Available from: http://www.ginasthma.org/.

2.4 Asthma management and treatment

Asthma treatment goal is to prevent and control asthma symptoms, reduce the frequency and severity of asthma exacerbations, and reverse airflow obstruction.[14,74,82] Recommendations in the treatment choices reflect the scientific fact that asthma is a chronic disorder with episodes of airflow limitation, cough, and mucus production.[80] Asthma medications are categorized into two classes: *quick-relief* medications which are taken as needed to achieve prompt reversal of acute pulmonary obstruction and relief of the accompanying bronchoconstriction (these medications are also known as acute rescue or reliever medications) and *long-term controller* medications which are taken daily on a long-term basis to achieve and maintain control of persistent asthma (these medications are also known as long-term preventive, maintenance, or controller medications). Patients with persistent asthma are in need of both classes of medication.[14,74]

An asthma attack or exacerbation could occur due to several triggers.[14] The typical symptoms of an exacerbation include dyspnea, wheezing, cough, and chest tightness.[14,74] The onset may be sudden, with a feeling of constriction in the chest and breathing difficulties.[14,74] Due to its potential risks, exacerbations should be managed as soon as their signs and symptoms are recognized.[14,74]

2.4.1 Relievers

These are quick-relief medications which are used as needed to treat acute asthma symptoms and exacerbations.[14,74]

- **Short-acting beta$_2$-agonists (SABA)**: examples include salbutamol, terbutaline, fenoterol, levalbuterol, metaproterenol, and pirbuterol. SABA are

considered the first line therapy for the relief of acute asthma symptoms, acute exacerbations and as pre-treatment for exercise-induced bronchoconstriction (see Figure 2.3.1).[14,74]

- **Short-acting anticholinergics**: the most frequently used is ipratropium bromide, which provides additive benefit to SABA in moderate and severe exacerbations.[14] It can also be considered as alternative bronchodilator for patients intolerant to inhaled beta$_2$-agonists.[14,74]
- **Systemic corticosteroids**: examples include prednisone, prednisolone, and methylprednisolone. Short courses of oral corticosteroids or parenteral corticosteroid solutions can be used for moderate and severe exacerbations to speed recovery and prevent exacerbation recurrence.[74]

2.4.2 Controller medications

Controller medications are daily-use long-term medications that help keep asthma symptoms under control. The most effective are those that reduce the underlying inflammation of asthma.[14,74]

- **Corticosteroids**: Corticosteroids are the most potent and effective anti-inflammatory medications currently available.[14,74,82] The main advantage of inhaled corticosteroids (ICS) in asthma management is their anti-inflammatory activity, which reduces the bronchial hyper-reactivity. ICS have fewer side effects than oral or systemic corticosteroids, and are considered the cornerstone therapy for persistent asthma management.[14,74] The most commonly used ICS are beclomethasone dipropionate, budesonide, ciclesonide, flunisolide, fluticasone propionate, mometasone furoate, and triamcinolone acetonide. Due to their high efficacy and low systemic levels when used as prescribed, ICS have superiority over any other single long-term controller medication.[74] After initiating the ICS therapy, the patients' symptoms improve in the first one to two weeks, with a maximum effect in 4 to 8 weeks.[83] ICS use is associated

with a decrease in asthma symptoms, emergency visits and hospitalizations for asthma, frequency and severity of exacerbations, mortality due to asthma and an improvement in lung functions and quality of life.[14,74]

Systemic corticosteroids are often used to achieve quick control of severe asthma symptoms. [14,74,83] Long-term oral corticosteroids therapy (tablets or syrup) are used only as needed in cases of uncontrolled severe persistent asthma (see Figure 2.3.1), and their discontinuation is recommended as soon as asthma control is achieved.[14,74,83]

- **Long-acting beta$_2$-agonists (LABA)**: Long-acting bronchodilators (mainly salmeterol and formoterol) are used alongside ICS for long-term control of asthma symptoms. [14,74] LABA have bronchodilation period of at least 12 hours after a single dose. [14,74] LABA should not be taken alone in asthma, as they do not have a chronic anti-inflammatory activity and their use as monotherapy showed a significant increase in asthma related deaths.[14,74] LABA and ICS combination is recommended for long-term control in moderate and severe persistent asthma (steps 3 to 6 in the stepwise approach for managing asthma, see Figure 2.3.1).[74] The LABA are also currently available in combination with ICS in a single ready-to-use inhaler (fluticasone/salmeterol and budesonide/formoterol). The combination of LABA and ICS is one of the preferred treatment choices when a low or medium dose of ICS fails to achieve the efficient control of asthma.[14] Their use in combination with an ICS allows asthma control at lower ICS doses, improves the lung functions, decreases SABA use and reduces exacerbations. [14,74]

- **Chromones**: Mild to moderate anti-inflammatory medications (e.g. cromolyn sodium and nedocromil). They are recommended as an alternative, but not preferred, medication for patients with mild persistent asthma (see Figure 2.3.1). They also can be used as preventive treatment prior to exercise or unavoidable exposure to known allergens.[74]

- **Leukotriene receptor antagonists** (LTRA): LTRA (e.g. zafirlukast and montelukast) are possible alternative therapies to low doses of ICS.[74] They are less effective than ICS and may be appropriate for initial controller treatment for patients who are unable or unwilling to use ICS, patients who experience intolerable side-effects from ICS or patients with concomitant allergic rhinitis.[14]

- **Methylxanthines**: Sustained-release theophylline is a mild-to-moderate bronchodilator mainly used as adjuvant to inhaled corticosteroids for prevention of nocturnal asthma symptoms.[74] It only has weak efficacy in asthma and reported several side-effects, which may be life-threatening at higher doses.[14,74]

- **Biologic-based therapy** (e.g. omalizumab mepolizumab, reslizumab)**:** Given that a large proportion of asthma symptoms are triggered by allergic reactions, immunoglobulin E (IgE), the antibody intimately involved in allergic responses, has been one target of therapy.[84] [85]Omalizumab is a monoclonal antibody directed against IgE, and acts to prevent its ability to function with multiple cell types.[86,87] Omalizumab has been shown to be safe and effective for adults and children in the treatment of asthma.[88] Omalizumab has been available to patients with asthma for more than 10 years.[87] However, it is ineffective in some patients whose asthma remains uncontrolled, and these patients subsequently discontinue this therapy. Recently, the FDA has approved reslizumab, a new interleukin-5-antagonist monoclonal anti-body that is administered by I.V. infusion once every 4 weeks.[89,90]

2.5 Asthma during pregnancy

Asthma is considered one of the most frequent chronic diseases that affect pregnant women.[1,91] Asthma has a prevalence of 3.7% to 12% among pregnant women.[2-6,92] Furthermore, asthma during pregnancy is showing an overall increasing prevalence over

time.[2-6] Among the well-known asthma management guidelines that include pregnancy recommendations are: the GINA (2015)[14], British Thoracic Society[93], the NAEPP-EPR3[5] and the Canadian Thoracic Society guidelines (1999).[82] The NAEPP published explicit and detailed guidelines for asthma management during pregnancy in 2004. The following sections will focus on the most updated pregnancy guidelines; the NAEPP (2004) and parts of GINA (2015).[5,14]

2.5.1 Impact of pregnancy on asthma

Among asthmatic pregnant women, approximately one third of the patients suffer from worsening of their asthma symptoms, one third experiences some improvements, and one third has their asthma symptoms remaining unchanged.[94-96] In a meta-analysis of 14 studies, the distribution of changes in asthma symptoms during pregnancy was in agreement with the rule of thirds, however in some studies this distribution may be still population-dependent.[97] Importantly, since the course of asthma can change during pregnancy, pregnant women need a closer follow up and rapid therapy adjustments to achieve optimal control of symptoms.[7,96,98,99]

It has been shown that more severe asthma is more likely to worsen during pregnancy.[4,10,100,101] The fewest symptoms occur after 37 weeks and nearly 75% of women return to their pre-pregnancy status within 3 months after delivery.[102] Moreover, the change in course of asthma tends to be consistent during successive pregnancies and exacerbations during delivery are relatively rare.[5,98,102] Asthma is an extremely variable disease, and a number of physiologic changes occur during pregnancy, which could worsen or improve asthma.[1,5,102,103]

During the pregnancy period, the uterus expands and causes elevation of the diaphragm by 4-5 centimeters, resulting in a decrease in lung functional residual capacity (FRC) of 10%-25%.[104] This decrease does not usually cause significant changes in the forced vital capacity, peak expiratory flow rate, or forced expiratory volume in 1 second (FEV1).[104,105] Minute ventilation (V_E) may be elevated as much as 50% by the third trimester of pregnancy due to progesterone-driven increases in tidal volume and respiratory

rate.[104] Concomitantly, oxygen consumption can increase up to 35%.[104,106] Estrogen changes in pregnancy affect the upper respiratory tract and the airway mucosa resulting in mucosal edema, hypersecretion and capillary congestion.[104,105] Differing levels of certain circulating hormones in an individual patient during pregnancy may account in part for why some patients' asthma worsens while other patients' asthma is improved.[102,104]

A physiological suppression of the immune system occurs during pregnancy, primarily to protect the fetus from the mother when paternally originated antigens are expressed.[4] Several cellular processes arise and both regulatory T cells (Tregs) and natural killer (NK) cells appear to play important roles.[4] It has been shown that regulatory NK cells and Tregs inhibit fetal attack of maternal NK and T cells.[4] A shift towards a T-helper cell type 2 (Th2)-predominant inflammatory occurs during pregnancy, with a simultaneous Tregs suppression of Th1 cell-induced fetal rejection.[107] Asthma is also categorized as a Th2-predominant inflammatory state.[4]

Both interleukin-4 (IL- 4) and interferon γ (IFN-γ) synthesizing T lymphocytes increase in pregnant women with uncontrolled asthma in comparison to those without asthma.[108] Moreover, pregnant women with asthma had a 20-fold increase in IFN-γ-producing T cells compared with non-pregnant patients having the same level of asthma severity.[102] These results imply that cellular responses change with the variation in the severity and control of asthma (mainly poorly controlled asthma).[102] They also demonstrate the heterogeneous response of the immune system in asthmatic women during pregnancy, and partially explain why asthma worsens, improves or remains unchanged during pregnancy.[102,105]

2.5.2 Impact of asthma on pregnancy

The critical effect of asthma during pregnancy on the fetal development is demonstrated through the possibility of inducing hypoxia combined with acute or compensated respiratory acidosis, besides an acute respiratory alkalosis that decreases the placental blood flow, increases systemic and pulmonary vascular resistance, and decreases cardiac output.[109,110] In cases of fetal lack of oxygen, the oxygen extraction rate by fetal

tissues increases and could lead to long-term effects of hypoxia as intrauterine growth retardation, preterm birth, neonatal hypoxia or perinatal morbidity and mortality.[110-113]

Pregnancy outcomes of asthmatic women compared to non-asthmatic women were examined in several studies, and results have shown increased risks in various adverse maternal and fetal outcomes among asthmatic mothers.[2,8-10,99] The risk of placental and maternal complications in pregnancy were examined in a recent meta-analysis.[8] The authors examined the association between maternal asthma and cesarean delivery, gestational diabetes, hemorrhage, placenta previa, placental abruption, chorioamnionitis, and premature rupture of membranes.[8] Compared to non-asthmatic women, maternal asthma was associated with a significantly increased risk of caesarean section (RR 1.31; 95% CI 1.22–1.39, I^2 = 90.8%), gestational diabetes (RR 1.39; 95% CI 1.17–1.66, I^2 = 88.4%), haemorrhage (antepartum: RR 1.25; 95% CI 1.10–1.42, I^2 = 71.3%; postpartum: RR 1.29; 95% CI 1.18–1.41, I^2 = 39.1%), placenta praevia (RR 1.23; 95% CI 1.07–1.40, I^2 = 0.0%), placental abruption (RR 1.29, 95% CI 1.14–1.47, I^2 = 44.8%) and premature rupture of membranes (RR 1.21, 95% CI 1.07–1.37, I^2 = 74.6%).[8] Furthermore, moderate-to-severe asthma significantly increased the risk of both caesarean section (RR 1.19, 95% CI 1.09–1.31, I^2 = 0.0%) and gestational diabetes (RR 1.19, 95% CI 1.06–1.33, I^2 = 65.5%), compared with mild asthma.[8] The limitations of the meta-analysis include: 1) there were fewer studies with prospective design, 2) in many studies, asthma was defined by self-report, 3) there was limited ability to account for the influence of some confounding factors such as socioeconomic status, smoking history, preexisting hypertension and BMI because this information was not included in most primary studies, and 4) although active management assessment was based on author clinical involvement, this does not equate to adequate control, limiting the ability to evaluate the potentially important effect of asthma control on the outcomes evaluated.

Preeclampsia was examined in another meta-analysis, which showed a significant increased risk of preeclampsia among mothers with asthma (RR 1.54; 95% CI 1.32 - 1.81, I^2 = 80.3%).[99] Adjustment for various covariates in six studies confirmed the effect of asthma on pre-eclampsia, as the adjusted odds of pre-eclampsia remained significantly increased in women with asthma compared with women without asthma.[99] A recently

published large population-based study using the Swedish Medical Birth Registry reported a significant increased risk of preeclampsia (aOR 1.15; 95% CI 1.06 - 1.24) and caesarean section (aOR 1.29; 95% CI 1.23 - 1.34) in pregnancies of asthmatic versus non-asthmatic women.[114] In a recent study conducted by our research group using the Quebec health databases, Blais et al. found that the risk of gestational diabetes was not associated with asthma severity or control, through comparing severe to mild asthma and uncontrolled to controlled asthma. Also, the risk of pregnancy-induced hypertension was not associated with asthma severity, but severe asthma was associated with an increased risk of caesarean delivery.[115] As being in the focus of this thesis, a summary of the evidence on maternal asthma and the risk of both congenital malformations and other perinatal outcomes are presented in the following subsections.

2.5.2.1 Maternal asthma and congenital malformations

Although it could be difficult to separate the effect of the disease – and its severity and control levels – from the effects of asthma medications, the whole body of evidence suggest that maternal asthma could significantly increase the risk of several adverse perinatal outcomes, including congenital malformations. A recent systematic review and meta-analysis by Murphy et al. examined the risk of congenital malformations, among other outcomes, in pregnant women with asthma.[2] The authors retrieved 16 publications and included 12 publications in their pooled meta-analysis. The authors also performed several sensitivity analyses separating prospective and retrospective studies and studies with and without active management of asthma.[2] There were four prospective cohort studies (all had active asthma management) and eight retrospective cohort studies (none had active management). In the primary analysis, asthma was associated with a significant increase in the risk of any congenital malformations (RR 1.11, 95% CI 1.02–1.21).[2] Sensitivity analysis showed that the effect was only significant in the retrospective cohort studies (without active management, RR 1.12, 95% CI 1.03–1.22), and not in the prospective cohort studies (with active asthma management, RR 1.40, 95% CI 0.54–3.59).[2] The power of the prospective studies was considerably low (10% power to detect an RR of 1.11).[2] A separate meta-analysis on the adjusted odds ratio from four retrospective studies

showed significant increased odds of congenital malformations (aOR 1.18, 95% CI 1.03–1.35).[2]

Major malformations were also examined through pooling results from four studies, showing increased risk of malformations that did not reach statistical significance (RR 1.31, 95% CI 0.57–3.02), however, there was significant heterogeneity between studies (I^2 = 70.9%, P < 0.1).[2] The authors also retrieved results on specific congenital malformations. The pooled meta-analysis of two studies demonstrated a significant increased risk of cleft lip and/or cleft palate among infants from women with asthma compared with infants from women without asthma (RR 1.30, 95% CI 1.01–1.68).[2] The limitations of the meta-analysis include: 1) there were fewer studies with prospective design, 2) asthma was defined by self-report in many studies, 3) reporting and recall bias in some studies, 4) the presence of significant heterogeneity in some analyses, and 5) the very large sample sizes in some of the retrospective studies could have resulted in heterogeneity that is overstated compared with traditional meta-analyses.

Exacerbations are potentially dangerous to the fetus as they can provoke maternal hypoxia combined with respiratory alkalosis, which could decrease the placental blood flow.[116,117] Hypoxia could cause abnormal development of the fetus,[118] and it has been found to be associated with an increased risk of cleft lip and palate in mice.[119] Exacerbations are common during pregnancy, reaching about 30% among pregnant women with severe asthma.[120] Asthma exacerbations were associated with congenital malformations in several studies.[2,117,121-123] In the meta-analysis by Murphy et al., a separate sensitivity analysis combining three studies on the effect of asthma exacerbations revealed an increased risk of congenital malformations, but did not reach statistical significance (RR 1.18, 95% CI 0.94–1.47).[2] Another sensitivity analysis on major congenital malformations from two studies revealed similar results (RR 1.26, 95% CI 0.95–1.67), with no significant heterogeneity between the studies (I^2 = 0%, P = 0.71).[2] However, there is a potential methodological and clinical heterogeneity that were not examined in the meta-analysis, which could have resulted from the variability in the study designs/risk of bias, and the variability in the patients' severity profiles. Moreover, the pooled result was heavily affected by the weight of one large study versus the second small one (13117 participants

versus 73 participants). In a recent study using the Quebec health databases, Blais et al. examined asthma exacerbations in a large representative cohort of 36,587 pregnancies of asthmatic women (publicly insured, privately insured and on social welfare assistance) and the risk of any and major congenital malformations.[117] The study showed that only severe maternal asthma exacerbations (i.e. requiring hospitalization) during the first trimester are associated with a significant increased risk of congenital malformations (aOR 1.64, 95% CI 1.02–2.64) and a non-significant increased risk of major malformations (aOR 1.70, 95% CI 0.95–3.02).[117]

2.5.2.2 Maternal asthma and other perinatal outcomes

Perinatal outcomes reported to be significantly increased among newborns of asthmatic women versus non-asthmatic women include SGA, LBW, preterm delivery, transient tachypnea of the newborn, neonatal hypoxia, and neonatal hyperbilirubinemia.[7,10,11,99,105] Furthermore, severe or uncontrolled asthma was associated with adverse perinatal outcomes in different observational studies, including perinatal mortality, IUGR, preterm birth, LBW.[5,94,117,124] On the other hand, it has been shown that women with well controlled asthma have little or no increased risk of adverse perinatal outcomes.[5,94,125,126] It is worth mentioning that the published evidence has been somehow conflicting, where large database studies reporting increased risks, while no significant increased risks found in smaller clinical prospective cohort studies. In general, the published studies varied substantially in terms of design and sample size.

Murphy et al. published a comprehensive systematic review and meta-analysis on maternal asthma and the risk of perinatal outcomes.[99] The outcomes examined included LBW, mean birthweight, SGA and preterm delivery.[99] The authors reviewed the evidence between 1975 and 2012 and included very large sample sizes of pregnant women (over 1,000,000 for LBW and over 250,000 for preterm delivery).[99]

In the meta-analysis of 11 studies, maternal asthma was associated with a significantly increased risk of LBW (defined as a birthweight < 2500 g) compared with women without asthma (RR 1.46, 95% CI 1.22 – 1.75).[99] In addition, the mean birthweight

of newborns of asthmatic mothers was 93 g lower than newborns of control mothers (95% CI -160 – -25 g). In a sensitivity analysis, the study design was acknowledged as a potential source of heterogeneity, where the prospective studies showing no effect of asthma (n = 3, RR 1.07, 95% CI 0.76 – 1.49), and the retrospective studies showing a significant effect (n = 8, RR 1.54, 95% CI 1.26 – 1.87).[99] These results were further confirmed in the active management sensitivity analysis (active management; RR 1.55, 95% CI 0.69 – 3.46, no active management; RR 1.50, 95% CI 1.23 – 1.82).[99]

In the same meta-analysis by Murphy et al., asthma was associated with a significant increased risk of SGA (11 studies: RR 1.22, 95% CI 1.14–1.31).[99] In the sensitivity analysis by study design, results obtained from retrospective and prospective studies were similar, and analysis of two studies adjusting for confounding factors showed a similar effect size of asthma on SGA (aOR 1.21, 95% CI 1.10 – 1.34).[99] Maternal asthma was associated as well with a significant increased risk of preterm delivery – defined as delivery prior to 37 weeks of gestation – (RR 1.41, 95% CI 1.23–1.62). Sensitivity analysis on confounding factors revealed similar results (4 studies: aOR 1.38, 95% CI 1.24 – 1.53). Sensitivity analysis on the presence or absence of active management revealed that a significant effect is present only among studies with no active management of asthma (RR 1.50, 95% CI 1.28 – 1.75).[99] Moreover, asthma was also associated with an increased risk of preterm labour – defined as premature uterine contractions prior to 37 weeks of gestation – (RR 1.71, 95% CI 1.14–2.57).[99]

It is worth noting that the majority of asthmatic women included in that systematic review had asthma of mild severity, and consequently the effect sizes of the observed risks could be larger in other subgroups of asthmatic women, such as moderate and severe asthma, uncontrolled asthma, and asthmatic women experiencing exacerbations during pregnancy. In fact, several studies have reported increased perinatal risks with the increase in asthma severity or the decrease in asthma control.[4,117,127]

In the recent study by Rejno et al. using the Swedish National Birth Registry, the authors examined the associations between maternal asthma and several birth and post-partum outcomes, namely birth weight, gestational age, SGA, large for gestational age (LGA), Apgar at 5 minutes, and asphyxia/hypoxia.[114] The authors adjusted for several

maternal confounding characteristics and examined the separate effects of both asthma severity and asthma control.[114] The study found a significant association between asthma and birth weight of 2000-3499 grams (30 % increased odds) as compared to ≥3500 grams, and SGA (OR 1.23, 95% CI 1.13–1.33).[114] Regarding asthma severity, compared to mild maternal asthma, moderate to severe maternal asthma was associated with an increased risk of SGA (OR 1.71, 95% CI 1.34–2.17) and birth weight 2000-3499 grams compared to ≥3500 grams. Uncontrolled asthma was associated with a significant increased risk of giving birth in week 37–38 (OR 1.29, 95% CI 1.07–1.56) among women with moderate to severe asthma but not among those with mild asthma.[114]

Several mechanisms were postulated to explain the observed increased perinatal risks among pregnant asthmatic women as demonstrated in different studies, including: 1) hypoxia and other physiologic consequences of poorly controlled asthma; 2) medications used for asthma treatment; and 3) other factors associated with asthma but not caused by the disease or the treatments (e.g. abnormal placental function).[99,102,105]

2.5.2.3 Pharmacologic treatment of asthma during pregnancy

The GINA 2015 guidelines states that: "[…] Although there is a general concern about any medication use in pregnancy, the advantages of actively treating asthma in pregnancy markedly outweigh any potential risks of usual controller and reliever medications".[14] Consequently, the use of asthma medications is justified even when their safety has not been clearly proven. The GINA guidelines propose a strategy where the treatments are recommended based on the lowest effective dose that provides adequate control of asthma symptoms.[14] Similarly, the US NAEPP guidelines for the management of asthma during pregnancy (published on 2005) states that it is safer for the pregnant mothers to be treated with asthma medications than to have asthma symptoms or exacerbations.[5]

According to the US NAEPP guidelines for managing asthma during pregnancy, asthma control is defined as minimizing asthma symptoms during the day or night, minimizing asthma exacerbations, achieving no limitations on daily activities, maintenance of normal pulmonary function, minimal use of SABA, and minimizing the medications side effects.[5] Differences exist in the treatment steps between asthmatic pregnant and non-

pregnant women, obviously for the fact that among pregnant women the treatments should maintain control of asthma symptoms not only for the health and quality of life of the patient, but also to maintain a healthy fetal growth throughout the gestation period.[5,74] In the NAEPP guidelines, the stepwise approach for the pregnant women is ordered into 4 steps, instead of 6 steps for the non-pregnant patients (see Figure 2.3.1 and Table 2.5). [5,74] Other differences are: 1) the use of zileuton in steps 3 and 4 (moderate persistent asthma) among non-pregnant patients, 2) use of omalizumab for patients who have allergies in steps 5 and 6 (severe persistent asthma) among non-pregnant patients, and 3) the recommendations for making repeated attempts to reduce systemic corticosteroid levels in pregnant patients suffering from severe persistent asthma (step 4) (see Figure 2.3.1 and Table 2.5).[5,74]

The US NAEPP guidelines for managing asthma during pregnancy classified the severity of asthma into 4 categories: 1) mild intermittent, 2) mild persistent, 3) moderate persistent, and 4) severe persistent (similar to adult non-pregnant asthmatics, Table 2.3.1). [5] With the purpose of achieving the desired control of asthma symptoms, physicians use the stepwise approach to manage asthma during pregnancy (see Table 2.5).[5] For example, in the case of mild intermittent asthma, a SABA is used as needed to control asthma symptoms, which is typically sufficient for this level of severity. If the patient's symptoms are relieved and pulmonary functions normalized, SABA should be continued only as needed for no more than twice per week.[5] SABA are also prescribed for patients experiencing exercise-induced bronchospasm, shortly before exercise.[5,74] Salbutamol (also referred to as albuterol) is the preferred SABA due to its safety profile. Indeed, salbutamol is one of the most studied asthma medications with an ample quantity of efficacy and safety evidence during pregnancy.[5,14] SABA are used as well in persistent asthma as quick relief medications.[5] However, the use of SABA more than 2 times per week in intermittent asthma is an indicator of uncontrolled asthma, which may require initiating or increasing a controller therapy.[5,14]

Table 2.5 Stepwise Approach for Managing Asthma during pregnancy and lactation: Treatment

Classify Severity: Clinical Features Before Treatment or Adequate Control		**Medications Required to Maintain Long-Term Control**
Symptoms/Day **----------** **Symptoms/Night**	**PEF or FEV_1** **----------** **PEF Variability**	**Daily Medications**
Step 4 Severe Persistent		
Continuous ---------- Frequent	≤60% ---------- >30%	Preferred treatment: - High-dose inhaled corticosteroid AND - Long-acting inhaled $beta_2$-agonist AND, if needed, - Corticosteroid tablets or syrup long term (2 mg/kg per day, generally not to exceed 60 mg per day) (Make repeat attempts to reduce systemic corticosteroid and maintain control with high-dose inhaled corticosteroid.*) Alternative treatment: - High-dose inhaled corticosteroid* AND - Sustained release theophylline to serum concentration of 5–12 micrograms/mL
Step 3 Moderate Persistent		
Daily ---------- >1 night/week	>60%-<80% ---------- >30%	Preferred treatment: EITHER - Low-dose inhaled corticosteroid* and long-acting inhaled $beta_2$-agonist OR - Medium-dose inhaled corticosteroid* If needed (particularly in patients with recurring severe exacerbations): - Medium-dose inhaled corticosteroid* and long-acting inhaled $beta_2$-agonist. Alternative treatment: - Low-dose inhaled corticosteroid* and either theophylline or leukotriene receptor antagonist** If needed: - Medium-dose inhaled corticosteroid* and either theophylline or leukotriene receptor

		antagonist**
Step 2 Mild Persistent		
>2 days/week but <daily --- >2 nights/month	≥80% --- 20 to 30%	Preferred treatment: - Low-dose inhaled corticosteroid* Alternative treatment (listed alphabetically): cromolyn, leukotriene receptor antagonist** OR sustained-release theophylline to serum concentration of 5–12 micrograms/mL.
Step 1 Mild Intermittent		
≤2 days/week --- ≤2 nights/month	≥80% --- <20%	No daily medication needed. Severe exacerbations may occur, separated by long periods of normal lung function and no symptoms. A course of systemic corticosteroid is recommended.
Quick Relief All Patients		
Short-acting bronchodilator: 2-4 puffs **short-acting inhaled beta$_2$-agonist***** as needed for symptoms Intensity of treatment will depend on severity of exacerbation; up to 3 treatments at 20-minute intervals or a single nebulizer treatment as needed. Course of systemic corticosteroid may be needed. Use of short-acting inhaled beta$_2$-agonist*** >2 times a week in intermittent asthma (daily, or increasing use in persistent asthma) may indicate the need to initiate (increase) long-term-control therapy.		

Step Down

Review treatment every 1 to 6 months; a gradual stepwise reduction in treatment may be possible.

Step up

If control is not maintained, consider step up. First, review patient medication technique, adherence, and environmental control.

Goals of Therapy: Asthma Control

Minimal or no chronic symptoms day or night
Minimal or no exacerbations
No limitations on activities; no school/work missed
Maintain (near) normal pulmonary function
Minimal use of short-acting inhaled beta$_2$- agonist***
Minimal or no adverse effects from medications

* There are more data on using budesonide during pregnancy than on using other inhaled corticosteroids.

** There are minimal data on using leukotriene receptor antagonists in humans during pregnancy, although there are reassuring animal data submitted to FDA.

*** There are more data on using albuterol during pregnancy than on using other short-acting inhaled beta$_2$-agonists.

Source: National Asthma Education and Prevention Program. Managing asthma during pregnancy: recommendations for pharmacologic treatment; National Heart, Lung, and Blood Institute; 2005

SABA use in the management of asthma during pregnancy is widely endorsed due to the known selectivity of beta$_2$-agonists and their minimal systemic effects when inhaled.[5,14,24] Besides, SABA have a crucial role in the management of acute exacerbations during pregnancy, both home and hospital/clinic managed.[14,74] For the home management, a salbutamol inhaler is recommended and in the hospital or clinic management, salbutamol is usually given through a nebulizer. [14]

For persistent asthma management, ICS are the cornerstone therapy in the management of all types of persistent asthma during pregnancy. LABA are used in cases of moderate and severe persistent asthma (Table 2.5), in combination with low or medium dose inhaled corticosteroids. The choice between salmeterol and formoterol is not supported with sufficient data, so salmeterol is relatively preferred as it has been available in the markets for longer periods. [5]

LABA have been introduced in the 1990s as a major therapeutic development in the management of asthma.[128] LABA are used for patients with moderate and severe persistent asthma not fully controlled with inhaled corticosteroids alone. According to the guidelines of asthma management during pregnancy, there is only limited observational data on the use of LABA during pregnancy. [5] However, since the publication of the NAEPP guidelines, there has been several new studies investigating the maternal and fetal safety of LABA during pregnancy, which we will discuss in thorough details in the following sections (subsection 2.6.1.2 and 5.1).[7,129] Both salmeterol and formoterol are available in the markets in separate forms or in combinations with ICS. In animal models, both salmeterol and formoterol have shown fetal risks, with delayed fetal ossification and other adverse outcomes at high doses.[130]

The associations between ICS maternal use and perinatal outcomes were examined in several studies.[2,129,131] As being in the center of this thesis, we will present the summary

of the evidence on ICS and major malformations in a separate detailed subsection below. The other adverse perinatal outcomes associated with ICS maternal use were summarized in a few systematic reviews, the most comprehensive is by Breton et al..[129,131] Breton et al. reviewed 14 studies examining ICS and different perinatal outcomes (the latest study was published in 2007 and 6 more studies were published since that date [discussed below]).[131] Significant associations between ICS use during pregnancy and mean birth weight were found in two cohort studies. [132,133] In a study by Murphy et al. an increased mean birth weight in female newborns was observed compared with the babies of asthmatic women who did not use ICS.[132] Norjavaara and de Verdier reported a significant effect of ICS use on the mean birth weight among 1409 girls and 1559 boys compared to all girls and boys born in Sweden between 1995 and 1998.[133] No studies reported significant associations between ICS use during pregnancy and low birth weight. Regarding preterm delivery, only Perlow et al. reported a significant association between the risk of preterm delivery and ICS use during pregnancy.[40] Preterm delivery occurred significantly more often in women who used an ICS compared with women without asthma (cOR 4.0; 95% IC, 1.1–15.5).[40] No significant increased risk of either stillbirth or perinatal mortality among pregnant women using ICS was observed.[131] It is worth mentioning that this review identified only nine studies using a control group of women with asthma not using ICS during pregnancy (which could be considered the most appropriate control group).

More recently, a cohort study by our group examined the relationship between asthma controllers and preterm delivery, LBW and SGA.[134] Among the 7376 included pregnancies, 56.9% were exposed to ICS. Adjusted odds ratio revealed no increased risk of preterm delivery, LBW or SGA with ICS use at any dose (low: >0–62.5, >62.5–125, >125–250; moderate: >250–500; and high doses: >500 mcg/day of fluticasone equivalent).[134] Due to the presence of a trend of increased risk of some outcomes with ICS doses above 125 mcg/day (where confounding by asthma severity could have played a role), further evidence is needed to explain this trend. [134] It is difficult to establish whether any of the observed adverse events in the previously mentioned studies were attributable to the medications or the effect of uncontrolled asthma. Among the suggested effective ways to tackle this type of bias is to perform a study where two medications that have similar indications can be examined to compare their relative safety.

Very few comparative studies of ICS medications during pregnancy have been published. Dombrowski et al. found fewer hospital admissions for the triamcinolone group compared to those treated with beclomethasone and a lower trend for low-birthweight infants.[45] Bakhireva et al. found no significant differences in SGA or mean birth weight between users of beclomethasone, budesonide, or fluticasone and control groups of non-asthmatic patients and users of SABA.[44] Namazy et al. found no significant differences in the prevalence of SGA or mean birth weight between users of beclomethasone, budesonide, or fluticasone.[135] Importantly, these studies had limited sample sizes. Clifton et al. reported a significant reduction in birth weight and length centile in users of the fluticasone-salmeterol combination compared with budesonide but no significant difference when fluticasone was compared with budesonide.[136] In a recent large cohort study published by our group, Cossette et al. found no statistically significant differences in the prevalence of LBW, preterm delivery, and SGA between women exposed to fluticasone (n=3190) and those exposed to budesonide (n=608) during pregnancy.[137] This lack of difference suggests that upon becoming pregnant, women with well-controlled asthma before pregnancy do not have to consider switching to another ICS.[137]

The maternal use of SABA and LABA and the associated risks of adverse perinatal outcomes were examined in several studies.[102,129,131] In the first part of this thesis, we present a systematic review that summarizes the body of evidence on the impact of the use of inhaled SABA and LABA for the treatment of asthma during pregnancy on several perinatal outcomes, which are major and any congenital malformations, SGA, birth weight, LBW, gestational age and preterm delivery. As being part of the focus of this thesis, SABA and LABA use and the risk of major malformations will be elaborated in detail in the subsection 2.6.1. Major – and not any or all – malformations were chosen as the primary outcome in this thesis for two reasons: 1) major malformations are more relevant to our objectives – compared to minor malformations – as they represent the more severe and life-threatening cases, and 2) the likelihood of misclassifications is greater for minor versus major malformations.

Under-treatment is a major concern in the management of asthma during pregnancy. It is considered one of the most important causes of uncontrolled asthma during pregnancy,

leading to an increased risk of adverse maternal and perinatal outcomes through the potential increase in asthma exacerbations. [5,14] For the most part, under-treatment is caused by the non-adherence of asthmatic pregnant women to their asthma controller medications due to their fears of a potential harm to their fetus.[94] In a study using maternal interviews, one of the important reasons for non-adherence was the concern about medication use, specifically corticosteroids, which surpassed the concern of the potential risk of uncontrolled asthma.[94] Therefore, asthma education is a key component in the management asthma during pregnancy, particularly regarding the adverse effects of uncontrolled asthma on the newborn and other self-management strategies such as how to handle new or increased asthma symptoms. [5,14]

In an online survey among pregnant women 18 to 44 years old about asthma treatments, 39 % reported having discontinued or reduced it, and a third of them did so without their physician consultation.[138] Another study showed that among women who used asthma medications prior to pregnancy, SABA claims were reduced by 52% during pregnancy and ICS by 36%.[139] Corroborating these results, in a study published by our group using administrative health databases from Quebec, we found that nearly 50% of asthmatic women discontinued or reduced their use of ICS during pregnancy as compared to the dose taken prior to pregnancy.[101] These behaviors put the asthmatic pregnant women at risk of exacerbations and inadequate control during pregnancy.

Data from the Netherlands between 2004 and 2009 showed a significant decrease in the filled prescriptions for asthma during the first trimester compared to 3 months prior to pregnancy.[140] In particular, prescriptions filled for long-acting bronchodilators and combination therapies (38.2% of pregnancies with 3 prescriptions in the year prior to pregnancy did not use any asthma medication during the first trimester).[140] In another study, there was a prescriptions decline of 23% in ICS, 13% in SABA and 54% in oral corticosteroids during the first trimester.[141]

2.6 Asthma treatments and major congenital malformations

2.6.1 Beta$_2$-agonists and major congenital malformations

In the first part of this thesis, we present a systematic review (full manuscript in chapter 5, section 5.1) in which we aimed to summarize the existing human data on the impact of the use of inhaled SABA and LABA for the treatment of asthma during pregnancy on several perinatal outcomes, including major congenital malformations. The latest search for publications in that systematic review was performed on January 1, 2013. For the current chapter of the thesis, we updated our search for additional articles published since that date and until April 1, 2016. In this section (2.6.1) and in Table 2.6.A below we present the whole body of literature with no cut-off date until April 1, 2016.

We identified 15 studies that examined the association between the maternal exposure to beta$_2$-agonists and major congenital malformations (see Table 2.6.A). [24-30,35,36,38,43,44,51,142,143] Among those, eight examined beta$_2$-agonists as a group (SABA and/or LABA use),[25,26,35,36,43,44,142,143] eleven examined SABA separately,[24-30,38,51,142,143] and eight examined LABA separately.[25,27-30,51,142,143]

A major factor affecting the validity of the results in those studies is the type of the reference group. Using non-asthmatics as a reference group carries a potential risk to the study validity. As previously discussed in the sections above, asthma itself and its accompanying symptoms have been identified as significant risk factors for several perinatal outcomes, including congenital malformations. Therefore, confounding by indication (i.e. asthma itself) should be highlighted when interpreting the results of the identified studies. Therefore, better conclusions could always be withdrawn from studies comparing beta$_2$-agonists users against asthmatic non-users, as this comparison could more easily separate the effect of the medications from the disease itself. Importantly as well is the bias due to confounding by severity (i.e. the severity of asthma symptoms and exacerbations) which should be also taken into consideration. The reason is that this bias could affect all types of studies, even the ones that used a comparison group of asthmatics non-users.

2.6.1.1 Short acting beta$_2$-agonists and major congenital malformations

The following section summarizes the whole body of evidence on SABA use and major congenital malformations, partially presented in the systematic review (in chapter 5) and updated here until April 1, 2016. From the eleven studies that examined SABA separately from LABA, six were cohort studies and five were case-control studies (Table 2.6.A). The sample sizes varied between studies, ranging between 50 to over 35000 exposed pregnancies. In general, the number of exposed pregnancies were larger in the cohort studies. Only three studies used a reference group of asthmatic non-users.[24,27,30] Schatz et al. examined the safety of the maternal exposure to any SABA during pregnancy in relation to fetal development.[24] Major and minor congenital malformations, among other perinatal outcomes, were reported separately for anytime use during pregnancy and during the 1st trimester. Adjustment for asthma severity was based on medication requirement, and the use of other asthma medications. The authors also adjusted for smoking as a potential confounder and the analyzed data were prospectively gathered. Anytime and first trimester use of SABA during pregnancy were associated with a cOR of 0.65 and 0.74 respectively (p-value > 0.05 for both). [24] The study suffered some limitations, specifically the small sample sizes (9.7% power to detect the observed cOR of 0.74 in the 1st trimester) and using a medication requirement scale (that classify the asthma severity based only on the medications used and no other factors, such as asthma exacerbations) to adjust for the asthma severity which could be incomplete since other important factors better indicate the severity level, such as asthma exacerbations and hospitalizations for asthma. The authors concluded that SABA use is warranted but needs additional safety assessments. [24]

In a matched case-control study using the Health Improvement Network primary care database (THIN) in the United Kingdom, Tata et al. examined the risk of major malformations with maternal SABA use, both anytime during pregnancy and only during the 1st trimester.[27] The reference group was formed of pregnant asthmatic women not exposed to SABA. Adjusted odds ratios of 1.06 (95% IC, 0.94–1.19) with anytime during pregnancy and 1.01 (95% IC, 0.86–1.18) in the 1st trimester SABA use were reported. [27] The authors adjusted for several important potential confounders, including maternal

smoking, body mass index, socioeconomic status, maternal age and child sex, but did not adjust for maternal asthma severity nor asthma control.

The third study that used asthmatics non-users of SABA as a reference group is a large cohort study published earlier by our group.[30] The study comprised data from three health administrative databases from Quebec and examined SABA use during the first trimester, both as any exposure and as dose-per-week analyses (non-use: reference category, > 0 to 3 doses, > 3 to 10 doses, and > 10 doses per week). [30] Adjustments were performed for socio-demographic variables, maternal and fetal conditions and markers of asthma severity and control. SABA use in the 1st trimester was not found to be associated with an increased risk of major malformations (aOR 0.93; 95% CI, 0.80-1.08). Moreover, the analysis on SABA doses-per-week revealed no association with major and major system-specific malformations. [30] A remarkable strength in this study is its sample size, which included 7182 SABA users offering an 80% power to detect an aOR of 1.2. Other strengths include adjustment for asthma severity and control levels and avoidance of recall bias. The study limitations include the absence of medications dispensing data from hospitals, possible misclassification of SABA doses and the possibility of residual confounding due to incapacity to adjust for known risk factors, like maternal obesity and smoking.

We identified eight studies that used a reference group of non-asthmatic pregnant women or a mixed population of asthmatic and non-asthmatic women. [25,26,28,29,38,51,142,143] Kallen and Olausson used the Swedish Medical Birth Register in a cohort study and reported an increased risk of cardiovascular defects with salbutamol use (aOR=1.38, 95% CI=1.12-1.70), while no significant increased risk was found with terbutaline use (aOR=1.08, 95% CI=0.94-1.23).[51] In a recent report using a larger sample from the same database, Kallen reported a significant increased risk of both major malformations (aOR 1.10, 95% CI 1.04-1.10) and major cardiac malformations (aOR 1.17, 95% CI 1.07-1.29) with any SABA use in the 1st trimester.[28] However, both studies have used the general population as the reference group, which included non-asthmatic women. The authors as well did not exclude the possibility of the presence of a potential confounding by indication, which could explain the observed associations.[28,51]

In a series of case-control reports conducted by the National Birth Defects Prevention Study (NBDPS) in the US, some associations were observed between SABA use and major congenital malformations. [25,142,143] Munsie et al. reported a significant increased risk of separate cleft lip and cleft palate with aOR 1.79, 95% CI 1.07-2.99 and aOR 1.65, 95% CI 1.06-2.58, respectively.[143] Lin et al. reported cOR of 1.62 (calculated using provided data in the study) for the risk of gastroschisis, a major para-umbilical abdominal wall defect, with salbutamol and/or pirbuterol use.[25] Lin et al. also reported similar associations with major isolated selected defects in another study (see footnotes, Table 2.6.A).[142] However, there are major methodologic limitations in all of the NBDPS studies, namely: 1) the reference group contained non-asthmatic women, 2) potential recall bias as the interviews were completed 6 weeks to 2 years after the delivery and mothers of affected children may be more likely to report their exposures than mothers of controls, 3) not considering the frequency of the medication use or dose, 4) the inaccurate reporting of exposure time during pregnancy, and 5) possibility of selection bias as the response rates were consistently low. Given these major limitations, it is difficult to draw conclusions from those results. Two more studies reported no significant increased risks of major malformations, but have used only non-asthmatic pregnant women as the reference group which represents a major methodologic limitation.[29,38]

In summary, the evidence on maternal SABA use, and salbutamol in particular, have demonstrated adequate fetal safety results in several well designed cohort and case-control studies, which warrant their safe use for the management of asthma during pregnancy.

2.6.1.2 Long acting beta$_2$-agonists and major congenital malformations

The following section summarizes the whole body of evidence on LABA use and major congenital malformations, partially presented in the systematic review (in chapter 5) and updated here until April 1, 2016. We identified eight studies in the literature that examined the association between the maternal use of LABA and the risk of major congenital malformations (Table 2.6.A). [25,27-30,51,142,143] The number of exposed

pregnancies exceeded 100 in only three studies.[28-30] Four were cohort studies and four were case-control studies (Table 2.6.A). Only two of them used a reference group of asthmatic pregnant women in order to separate the effect of LABA from asthma disease itself,[27,30] among which one has found significant increased risk of major specific malformations.[30]

A cohort study published by our group examined all major and specific major malformations with LABA use during the 1st trimester.[30] An increased risk of major malformations was observed, though it did not reach statistical significance (aOR 1.31, 95% CI 0.74-2.31).[30] Moreover, Significant increased risks of major cardiac and major other and unspecified malformations were observed (aOR 2.38, 95% CI 1.11-5.10 and aOR 3.97, 95% CI 1.29-12.20 respectively).[30] However, the numbers of the exposed group and cases identified were small and there is a possibility of residual confounding due to the insufficient control for asthma severity and control levels. Also using a reference group of asthmatic pregnant non-users women, Tata et al. in a matched case-control study reported an aOR of 1.12 (95% CI 0.72-1.75) with the use of any LABA anytime during pregnancy and an aOR of 1.09 (95% CI 0.62-1.90) with the 1st trimester use.[27] However the study sample size was considerably low, preventing the inferring of solid conclusions. [27]

We identified six studies that examined LABA and major malformations while using a reference group of non-asthmatic pregnant women or a mixed population of asthmatic and non-asthmatic women. [25,28,29,51,142,143] Kallen and Olausson, using the Swedish Medical Birth Register, reported a nonsignificant increased risk of cardiovascular defects with salmeterol use (aOR=1.34, 95% CI=0.96-1.88) and formoterol use (aOR=1.07, 95% CI=0.63-1.82).[51] In a more recent report using the same registry, Kallen reported an aOR=1.08, 95% CI=0.96-1.22 for all major malformations and aOR=1.12, 95% CI=0.92-1.36 for major cardiovascular malformations.[28] However as mentioned earlier, both studies used the general population as the reference group. Vasilakis-Scaramozza et al. in a cohort study using the General Practice Research Database (GPRD) reported a cOR of 0.80, 95% CI=0.40-1.50 with any LABA use during the first trimester and using non-asthmatic pregnant women as a reference group.[29]

In the series of case-control reports from the NBDPS, Lin et al reported nonsignificant cORs of 1.33 and 1.97 for selected major malformations and all major

malformations respectively in two studies.[25,142] However, the studies suffered some major limitations as mentioned earlier, which included the major weakness of using non-asthmatic women in the reference group.

In summary, smaller body of evidence exists on LABA use during pregnancy with some evidence of specific congenital malformations increased risk. However, this risk might be attributable to the severity of asthma. In several studies we reviewed, methodologic limitations were common and the negative results obtained with low statistical power should be interpreted with caution as not to give a false impression of safety.

2.6.1.3 SABA & LABA and major congenital malformations

Examining the fetal safety of SABA and LABA combined as one group (i.e. any $beta_2$-agonist use) may not be as informative and conclusive as separating each class by itself, mainly due to the unique nature of each class and their different indications. However, some safety results could be obtained which we will cover in this subsection.

We identified eight studies – two cohort and six case-control studies – that examined the association between the maternal use of any $beta_2$-agonist and the risk of major malformations (Table 2.6.A).[25,26,35,36,43,44,142,143] The sample sizes of the identified studies were modest, except for one large cohort study.[43] Only two studies used a reference group of asthmatic pregnant women to separate the effect of SABA and LABA from asthma itself, and neither of them has found a significant association.[43,44] Bakhireva et al. examined the association between the maternal use of any $beta_2$-agonist anytime during pregnancy and perinatal outcomes, including major malformations in a prospective cohort study.[44] Comparing $beta_2$-agonists users against asthmatic ICS users, the authors didn't find any increased risk of malformations (calculated cRR = 0.95).[44] When compared against non-asthmatic pregnant women, the result was a staggering cRR = 13.0, which highlights the importance of using the appropriate reference group, in this case asthmatic women non users of ICS.[44] The prospective design limited the possibility of selection and recall bias and only 5% of the participants were lost to follow-up. However, self-reporting of the

maternal medication use (using telephone interviews conducted at enrollment, during pregnancy and 4 to 6 weeks after delivery) is considered a limitation, since maternal recall of medications use (frequency and doses) might not be highly accurate, leading to non-differential misclassification that underestimates the medications effect. Additionally, maternal exposures to SABA and LABA measured anytime during pregnancy (and not in the first trimester only, which is the most susceptible period for a teratogenic effect) can underestimate the effect of the exposure on major malformations. Schatz et al. examined the association between beta$_2$-agonists use anytime during pregnancy and adverse perinatal outcomes including major congenital malformations.[43] The authors reported a cRR of 1.0 with beta$_2$-agonists use compared to asthmatic non-users.[43] In both studies, the exposure timing (entire pregnancy) and combining SABA or LABA exposure prevents drawing solid conclusions.

The remaining six studies that examined the association between beta$_2$-agonists and major malformations used a reference group that includes non-asthmatic women, and all have shown significant increased risk of major congenital malformations (Table 2.6.A).[25,26,35,36,142,143] As previously discussed, these results are potentially confounded by the effect of asthma itself and asthma severity and control levels.

Garne et al. used the EUROmediCAT database to examine asthma medications effect in a case-malformed control study.[35] The authors reviewed the previously published studies and gathered the major malformations "signals" (i.e. significant associations) reported in those studies with maternal asthma medications use. Afterward, the authors conducted separate analysis on each association using their own data to either confirm or refute it. With any beta$_2$-agonist use (which comprised over 80% SABA users) significant increased risks of any major malformations, gastroschisis and cleft palate were observed (Table 2.6.A).[35] The authors concluded the study with an inaccurate and worrisome interpretation for beta$_2$-agonists use during pregnancy, ignoring the fact that the major limitation of the study is its reference group which was formed of asthmatics and non-asthmatics non users. We have discussed the major weaknesses of this study in a correspondence which was published in the same issue of the journal. We have included the correspondence in Chapter 5: Results.

Briefly, disentangling the effect of the medication from the disease is a challenging task that has to be appropriately addressed in both the design and the analysis of the study. Including non-asthmatic women in the reference group could have potentially overestimated the effect of the asthma medications. In the study by Garne et al., [35] 53% of asthmatic women treated with beta$_2$-agonists (86% using salbutamol) had no controller medications and were likely having undertreated uncontrolled asthma. Uncontrolled asthma itself, as discussed earlier, is associated with an increased risk of congenital malformations.[14] Due to this confounding by indication, an increased prevalence of malformations was found in the β_2-agonists group – corroborating results from previous case-control studies using similar reference groups. [25,26,143] SABA (salbutamol in particular) have shown fetal safety in several well designed cohort and case-control studies. The authors did not report the maternal characteristics of the women in the study (e.g. age, comorbidities, asthma exacerbations), which prevented the assessment of the comparability of the study groups. Other limitations include the lack of adjustment for important confounders such as socioeconomic status and asthma exacerbations, combining SABA and LABA under one exposure category, and multiple comparisons.

In a series of five case-control studies from the NBDPS, beta$_2$-agonists use 1 month prior to conception and during the 1st trimester was shown to be associated with significant increased risks of anomalous pulmonary venous return[36], esophageal atresia[142], cleft lip (without cleft palate)[143], major cardiac malformations[26] and gastroschisis.[25] As discussed earlier, these studies suffered major limitations that could have potentially affected the validity of the results, which included the key weakness of using non-asthmatic women in the reference group.

In summary, SABA and LABA combined as one group have shown some associations with major malformations, specifically in studies using non-asthmatic women in the reference group. The studies reviewed suffered major methodologic limitations as well. The results are neither informative nor conclusive as compared to separating each class by itself, due to the major differences in their indications for asthma management.

2.6.2 Inhaled corticosteroids and major congenital malformations

We identified - through our systematic search and review of the literature – thirteen published studies that examined the association between the maternal use of ICS during pregnancy and the risk of major congenital malformations, which we summarize in Table 2.6.B. [21,28,29,32-35,37,38,42-44,49] Eleven of which were cohort studies and two case-control studies (see Table 2.6.B). We have identified few reviews as well, but could not locate any meta-analysis of the published results. The systematic review and meta-analysis discussed earlier by Murphy et al. did not examine ICS use and major malformations, but rather examined any congenital malformations as an outcome.[2] Through aggregating results from 3 studies, any ICS use was not found to be associated with an increased risk of any malformations (RR 0.96; 95% CI 0.89-1.04).[2]

Among the thirteen studies located, six studies used a reference group of asthmatic women, which is a major step in separating the effect of the medications from the disease itself. [33,34,42-44,49] None of the six studies reported a significant association with ICS use (Table 2.6.B). The sample sizes of the studies were moderate to large, ranging between 150 and 1500 exposed pregnancies.

Charlton et al. in a recent cohort study used the GPRD database to examine the fetal safety of fluticasone.[33] Using a reference group of asthmatic pregnant women who used other ICS and stratifying the groups based on their asthma severity, the authors did not find a significant increased risk of major malformations with fluticasone use (aOR 1.10; 95% CI 0.50-2.30 among moderate asthmatics and aOR 1.20; 95% CI 0.70-2.00 among women with considerable to severe asthma).[33] The authors were able to adjust for important confounders, including alcohol consumption, smoking status, socioeconomic status, oral corticosteroid use and body mass index (BMI). A limitation in the study is that its objective was examining the fetal safety of fluticasone compared to other ICS, so that it became impossible to assess if there was a class effect for ICS. Another key weakness in the GPRD database is that the medication exposure data is based on the prescription issued, with no knowledge on either if it was dispensed or used. Additionally, a change in asthma severity cannot be captured in the database. Specifically, for pregnant women who had a change in their asthma severity and who did not see a physician to give them new prescriptions, such

change in asthma severity and control will not be captured in the database. There was also a possibility of residual confounding by asthma severity due to incomplete adjustment of severity levels.

In two studies conducted by our group using Quebec health administrative databases, Blais et al. examined ICS use during the first trimester and the risk of major congenital malformations.[34,49] The first study was a two-stage sampling study that allowed for the adjustment for important potential confounders collected through the mothers' medical charts, which included smoking status (0, 1–20, >21 cigarettes per day), alcohol use, illicit drug use, intake of multivitamins, intake of folic acid and exposure to irradiations or x rays.[49] Compared to a reference group of asthmatic pregnant women who were not exposed to ICS during the first trimester, ICS users were not found to be at a significant increased risk of major malformations at low and moderate doses (aOR 0.90; 95% CI 0.64-1.24 for >500 mcg/day of beclomethasone equivalent and aOR 0.56; 95% CI 0.22-1.43 for >500-1000 mcg/day of beclomethasone equivalent).[49] However, with the higher doses of >1000 mcg/day of beclomethasone equivalent a non-significant trend was found (aOR 1.67; 95% CI 0.56-5.03).[49] This result should be interpreted with caution however due to the small number of cases reported.

In the second study by Blais et al., a further investigation into the safety of high doses of ICS was conducted.[34] Data from 3 health databases were linked and a cohort of 13280 pregnancies was formed. ICS doses were categorized into 0, > 0-1000 and >1000 mcg/day of beclomethasone equivalent. Compared to pregnant asthmatic users of medium doses of ICS (> 0-1000 mcg/day), users of high doses of ICS (>1000 mcg/day) were found to be at a higher risk of major malformations which did not reach statistical significance (aOR 1.67; 95% CI 0.91-3.06).[34] Of note, when the same analysis was performed with all malformations as the outcome of interest, the results reached statistical significance (aOR 1.66; 95% CI 1.02-2.68).[34] The results of both studies however should be interpreted in the light of the observed higher proportion of women with markers of severe and uncontrolled asthma among users of high doses of ICS, which could result in residual confounding and overestimation of the effect of ICS on congenital malformations.

Bakhireva et al. examined the association between maternal use of any ICS anytime during pregnancy and perinatal outcomes, including major malformations in a prospective cohort study.[44] Compared to non-asthmatic pregnant women, the authors found a significant increased risk of major malformations (cRR 13.7, $P < 0.05$).[44] However, the association was greatly attenuated when ICS users were compared to asthmatic ICS non users (cRR 1.10, $P > 0.05$), which highlights the importance of using the appropriate reference group in similar studies to accurately assess the effect of the medication separated from the effect of asthma itself. The prospective design limited the selection and recall bias but self-reporting of the medication use was a major limitation, since maternal recall of medications use (frequency and doses) might not be highly accurate, leading to non-differential misclassification that underestimates the medications effect. Schatz et al. in another study examined the association between ICS use anytime during pregnancy and major congenital malformations.[43] The authors used a reference group of asthmatic non-users and reported a non significant cRR = 1.0. However, ICS use was examined anytime through the entire period of pregnancy and no adjustment was performed on any potential confounder.

Dombrowski et al. in a randomized controlled trial compared the efficacy of inhaled beclomethasone (400-500 mcg/day) to oral theophylline (400 to 800 mg/day) for the prevention of asthma exacerbations requiring medical intervention.[42] Despite the randomization, the small sample sizes have resulted in some observable differences in the baseline characteristics between the groups. The study concluded that the treatment of moderate asthma with inhaled beclomethasone versus oral theophylline led to similar rates of asthma exacerbations and similar obstetric and perinatal outcomes.[42] Six cases of major malformations were observed among the beclomethasone group and five cases among the theophylline users (cRR 1.20; 95% CI 0.40-3.80).[42]

We identified seven studies that used a reference group of non-asthmatic pregnant women or a mixed population of asthmatic and non-asthmatic women. [21,28,29,32,35,37,38] Kallen et al. in a case control study using the Swedish Medical Birth Register found no significant increased risk of major malformations with ICS use early during pregnancy (aOR=1.05, 95% CI=0.82-1.34).[21] In a recent report using the same registry, Kallen

reported a significant increased risk of major malformations (aOR=1.08, 95% CI=1.01-1.16) with ICS use in the first trimester, and a nonsignificant increased risk of major cardiac malformations (aOR=1.11, 95% CI=0.99-1.25).[28] The general population was used as a reference group and there was a potential confounding by indication that could explain the results.

Two large cohort studies have used the Danish National Registries to examine the association between ICS use and major malformations, specially orofacial clefts.[32,37] The largest was a study by Hviid et al that examined ICS use and cleft palates and cleft lips over a 12 year period in Denmark.[37] The authors collected data on 7421 users of ICS and adjusted for several important potential confounders, including level of education, socioeconomic status, maternal comorbidities and the maternal exposure to other medications. The study did not find a significant increased risk of neither cleft palate only (aOR=0.94, 95% CI=0.30-2.92) nor cleft lip with cleft palate (aOR=0.75, 95% CI=0.34-1.68).[37] The second study included 1223 users of ICS and reported unadjusted cOR=1.02, 95% CI=0.77-1.34 for all major malformations and unadjusted cOR=0.47, 95% CI=0.07-3.34 for oral clefts.[32] Both studies however used non-asthmatics among their reference group.

Vasilakis-Scaramozza et al. in a cohort study using the GPRD database reported a cOR of 1.10, 95% CI=0.90-1.40 with any ICS.[29] The study did not adjust for any potential confounders and restricted the reference group to only non-asthmatic pregnant women, which is a major methodologic limitation. The study described earlier by Garne et al. investigated also the safety of ICS use during pregnancy, using the EUROmediCAT database in a case-malformed control study.[35] The study did not find any significant increased risk of any major malformations or specific major malformations.

We have discussed the major weaknesses of this study in a correspondence published in the same issue of the journal. We have included the correspondence in Chapter 5: Results. Briefly, asthmatic women treated with beta$_2$-agonists had no controller medications and were likely having uncontrolled asthma. On the other hand, the ICS group was likely including women who were appropriately controlled due to the beneficial effect of ICS. Due to this confounding by control level, an increased prevalence of malformations

was found in the beta$_2$-agonists group and not found among the ICS group (even showing protective effects in some instances). No description on the maternal characteristics of the women in the study was reported (e.g. asthma exacerbations, hospitalizations for asthma and oral corticosteroids use), which prevented the assessment of the comparability of the exposure groups.

Of note, we have located some case-control studies conducted by the NBDPS in the US which examined the fetal safety of ICS among other asthma treatments. [25,26,36,142,143] However, ICS were combined with other anti-inflammatories (e.g. cromolyn, montelukast, nedocromil) in all of the located studies, which prevented a valid assessment of the separate effect of ICS use during pregnancy and gave rise to impractical and inadequate results that are difficult to interpret.

In summary, evidence on maternal ICS use during pregnancy has demonstrated sufficient fetal safety results in several studies. However, evidence might be still lesser for high doses of ICS which have their effect possibly confounded by asthma severity and control levels. In general, ICS are recommended to be safe for the management of asthma during pregnancy.

2.6.3 LABA-ICS combination and major congenital malformations

Despite being used for many years in the treatment of asthma during pregnancy, LABA-ICS combination therapy is one of the least studied treatment regimens. An explanation for such small body of knowledge is that researchers have focused on each medication (i.e. LABA and ICS) separately in an effort to tease out the effect of each medication on congenital malformations. However, women are usually treated with more than one single medication, making the comparison between different treatment regimens more relevant to the routine clinical practice and more useful for researchers, physicians and patients. Moreover, comparing treatment alternatives that have similar indications is one of the most effective methods in reducing and minimizing confounding by indication. Through our literature search, we have located three studies – two cohort studies and one case-control study – that investigated the association between LABA-ICS combination use

during pregnancy and the risk of major malformations.[28,33,35] A summary of the retrieved studies is presented in Table 2.6.C. One study[28] had a large number of exposed pregnancies (over 8000) and the other two were of small to moderate sample sizes. Another article by Eltonsy et al. can be located which is part of this thesis and not included in Table 2.6.C.

Among the three studies retrieved, only one study used the more appropriate reference group of asthmatic pregnant women.[33] Charlton et al. in a cohort study used the GPRD database to examine the fetal safety of fluticasone-salmeterol combination.[33] Using a modified asthma severity index developed by our team[144], the authors classified the asthmatic pregnancies into moderate asthma (177 combination therapy users) and considerable to severe asthma (1032 combination therapy users). Asthmatic users of ICS other than fluticasone were used as a reference group. The study did not find a significant increased risk of major malformations with the use of combination therapy (aOR 1.30; 95% CI 0.50-3.20 among moderate asthmatics and aOR 1.10; 95% CI 0.60-2.00 among women with considerable to severe asthma).[33] Adjustment for important confounders was performed, including alcohol consumption, smoking status, socioeconomic status, and BMI. Importantly however, the multivariate models used in the statistical analysis of the results above did not account for the presence of repeated measures (i.e. when 1 woman contribute more than 1 pregnancy into the cohort). The authors performed a sensitivity analysis restricted to the first pregnancy from each woman and the results changed drastically, especially among moderate asthmatics where the previously observed association was inversed (aOR 0.70; 95% CI 0.20-2.90 among moderate asthmatics and aOR 1.20; 95% CI 0.60-2.30 among women with considerable to severe asthma).[33] Moreover, since the objective of the study was to investigate the fetal safety of fluticasone (and its combination with salmeterol), it became difficult to generalize the results to other LABA-ICS combinations (e.g. budesonide-formoterol). Also, a key weakness in the GPRD is that the medication exposure data is based on the prescription issued, with no knowledge on either if it was dispensed or used. Data on the change in severity that were not accompanied by an issued prescription will not be captured. Finally, there was also a possibility of residual confounding by asthma severity due to incomplete adjustment of severity levels.

The other two studies that examined LABA-ICS combination therapy used a reference group that includes non-asthmatic women. The recent study by Kallen using the Swedish Birth Registry examined LABA-ICS combination therapy and found no statistically significant association between their maternal use during the first trimester and major malformations (aOR 1.07; 95% CI 0.95-1.21) or major cardiac malformations (aOR 1.01; 95% CI 0.81-1.25).[28] The second study by Garne et al. used the EUROmediCAT database to examine the maternal exposure to any LABA-ICS combination during the first trimester and major malformations.[35] No significant association was found between LABA-ICS and any major malformations, but a more than three folds significant increased risk of esophageal atresia was observed (aOR 3.63; 95% CI 1.26-10.42).[35] This increase could be due to the asthma and its severity, especially that the combination therapy is usually indicated in cases of moderate to severe persistent asthma, or it could be due to chance alone. The weaknesses of the study were discussed in a correspondence included in this thesis in Chapter 5: Results.

In summary, very small body of evidence exists on the fetal safety of the combination of LABA-ICS as a treatment regimen, despite their wide use among asthmatic women and the fact that LABA should be prescribed only in combination with ICS. The three studies retrieved suffered from methodologic limitations that prevent an accurate assessment of the associated risks. Given these facts, more evidence on the fetal safety of LABA-ICS and similar treatment regimens is clearly necessary.

Table 2.6.A. Studies Investigating the Association between Beta$_2$-agonists Use during Pregnancy and Major Congenital Malformations

Study ref.	Design	Source of data	Exposure Timing	Users of ß$_2$-agonists			Non-users of ß$_2$-agonists			Effect	
				Type of ß$_2$-agonists	n*	Major congenital malformation (%)	Definition	n*	Major congenital malformation (%)	OR or RR	95% CI or (p-value)
SABA and/or LABA use: cohort studies											
Bakhireva et al. (2005)	Cohort	Tel. interviews & medical charts	Entire pregnancy	Any	103	3.9	Non asthmatics	303	0.3	cRR 13.0	NA
				Any	103	3.9	Asthmatics ICS users [a]	438	4.1	cRR 0.95	NA
Schatz et al. (2004)	Cohort + RCT	Medical charts & interviews	Entire pregnancy	Any	1,828	2.0	Asthmatics non users [bcd]	295	2.0	cRR 1.0	(P >0.05)

Study ref.	Design	Source of data	Exposure Timing	Type of ß$_2$-agonists	Cases		Controls		Definition of non-users of ß$_2$-agonists	Effect	
					Users of ß$_2$-agonists	Non-users of ß$_2$-agonists	Users of ß$_2$-agonists	Non-users of ß$_2$-agonists		OR or RR	95% CI or (p-value)
SABA and/or LABA use: case-control studies											
Garne et al. (2015)	Case-Malformed Control	EUROmediCAT database (Birth registry, medical records & self reports)	1st trimester	Any	264	16539	592[t]	43232	Asthmatics and non-asthmatics non users	**aOR = 1.23**	**1.05, 1.46**
				Any	264	16539	97[u]	9481	Asthmatics and non-asthmatics non users	**aOR = 1.46**	**1.10, 1.93**
				Any	28	1364	592[t]	43232	Asthmatics and non-asthmatics non users	**aOR = 1.63[r]**	**1.05, 2.52**
				Any	28	1364	97[u]	9481	Asthmatics and non-asthmatics non users	**aOR = 1.97[r]**	**1.19, 3.25**
				Any	19	596	592[t]	43232	Asthmatics and non-asthmatics non users	**aOR = 1.89[z]**	**1.12, 3.20**
				Any	19	596	97[u]	9481	Asthmatics and non-asthmatics non users	**aOR = 3.04[z]**	**1.53, 6.06**
Van Zutphen et al. (2015)	Case Control	Registry, medical records & self reports	1 month prior conception + 1st trimester	Any	8	206	194	7912	Asthmatics and non asthmatics non users	**cOR = 2.3[v]**	**1.10, 4.80**
Lin et al. (2012)	Case Control	Registry, medical records & self reports	1 month prior conception + 1st trimester	Any[f]	10	168	NA	NA	Asthmatics and non asthmatics non users	**aOR = 2.39[o]**	**1.23, 4.66**

Munsie et al. (2011)	Case Control	Registry, medical records & self reports	1 month prior conception + 1st trimester	Any[f]	20	570	114	6207	Asthmatics and non-asthmatics non users	**aOR = 1.77[q]**	**1.08, 2.88**
			1 month prior conception + 1st trimester	Any[f]	26	887	114	6207	Asthmatics and non-asthmatics non users	aOR = 1.53[r]	0.99, 2.37
			1 month prior conception + 1st trimester	Any[f]	17	1114	114	6207	Asthmatics and non-asthmatics non users	aOR = 0.78[s]	0.46, 1.31
			2nd & 3rd trimesters	Any[f]	7	570	58	6207	Asthmatics and non-asthmatics non users	aOR = 1.26[q]	0.57, 2.80
			2nd & 3rd trimesters	Any[f]	4	887	58	6207	Asthmatics and non-asthmatics non users	aOR = 0.49[r]	0.18, 1.36
			2nd & 3rd trimesters	Any[f]	6	1114	58	6207	Asthmatics and non-asthmatics non users	aOR = 0.59[s]	0.25, 1.38
Lin et al. (2009)	Matched Case Control 1:2	Registry, medical records & tel. interviews	1 month prior conception + 1st trimester	Any[f]	22	443	22	965	Asthmatics and non asthmatics with no Rx	**aOR = 2.20[w]**	**1.05,4.61**
Lin et al. (2008)	Case Control 1:11	Tel. interviews& Rx DB	1 month prior conception + 1st trimester	Any[g]	17	358	96	3,932	Asthmatics and non-asthmatics non users [g]	**aOR = 2.06[z]**	**1.19,3.59**

SABA only: cohort studies

Study ref.	Design	Source of data	Exposure Timing	Users of ß2-agonists			Non-users of ß2-agonists			Effect	
				Type of ß2-agonists	n*	Major congenital malformation (%)	Definition	n*	Major congenital malformation (%)	OR or RR	95% CI or (p-value)
Kallen (2014)	Cohort	Swedish Registers (Medical birth, Congenital malformation and Hospital discharge)	1st trimester (early pregnancy, usually 10-12 weeks)	Any	35453	3.41	General population	NA	NA	**aOR = 1.10**	**1.04,1.10**
				Any	35453	1.22	General population	NA	NA	**aOR = 1.17[w]**	**1.07,1.29**
Vasilakis-Scaramozza et al (2013)	Cohort	General Practice Research Database (GPRD)	180-335 days before LB, 70- 225 SB	Any	7061	3.10	Non asthmatics non users	15840	2.78	cOR = 1.10	0.90,1.30

Eltonsy et al. (2011)	Cohort	Quebec administrative DB	1st trimester	Any	7,182	5.7	Asthmatics non users[g]	5,935	5.9	aOR = 0.93	0.80,1.08
				Any (>0–3 doses/week)	3,420	6.1				aOR = 1.00	0.83, 1.20
				Any (>3–10 doses/week)	2,102	5.5				aOR = 0.84	0.67, 1.06
				Any (>10 doses/week)	1,660	5.2				**aOR = 0.68**	**0.48, 0.95**
Kallen et al. (2007)	Cohort	Swedish Registers (Medical birth, Congenital malformation and Hospital discharge)	1st trimester	Salbut	NA	NA	General population	NA	NA	**aOR = 1.38[h]**	**1.12,1.70**
				Terbut	NA	NA		NA	NA	aOR = 1.08[i]	0.94,1.23
Schatz et al. (1997)	Cohort	Daily diary cards for medications completed by patients, tel. interviews & medical charts	Entire pregnancy	Any [e]	667	3.7	Non asthmatics	823	6.2	cRR 0.60	(P >0.05)
			1st trimester	Any [e]	488	4.3	Non asthmatics	1,000	5.6	cRR 0.77	(P >0.05)
Schatz et al. (1988)	Cohort	Questionnaire for patients identification, confirmed clinically + Self-Diary to report use of SABA & medical records (for perinatal outcomes)	Entire pregnancy	Any [bfj]	259	3.9	Non asthmatics	295	6.4	cOR = 0.61	(P >0.05)
			1st trimester	Any [bfj]	180	3.9	Non asthmatics	295	6.4	cOR = 0.61	(P >0.05)
			Entire pregnancy	Any [bfj]	259	3.9	Asthmatics non users[g]	101	6.0	cOR = 0.65	(P >0.05)
			1st trimester	Any [bfj]	180	3.9	Asthmatics non users[g]	172	5.3	cOR = 0.74	(P >0.05)
			Entire pregnancy	Any [bfj]	259	3.5	General population	1,999,254	3.0	cOR = 1.17	NA

SABA only: case-control studies

Study ref.	Design	Source of data	Exposure Timing	Type of ß2-agonists	Cases		Controls		Definition of non-users of ß2-agonists	Effect	
					Users of ß2-agonists	Non-users of ß2-agonists	Users of ß2-agonists	Non-users of ß2-agonists		OR or RR	95% CI or (p-value)
Lin et al. (2012)	Case Control	Registry, medical records & self reports	1 month prior conception + 1st trimester	Salbut	77	2776	139	6587	Asthmatics and non asthmatics non users	cOR = 1.31[P]	NA
				Pirbuterol	3	2850	3	6723	Asthmatics and non asthmatics non users	cOR = 2.36[P]	NA

Munsie et al. (2011)	Case Control	Registry, medical records & self reports	1 month prior conception + 1st trimester	Salbut	18	570	101	6207	Asthmatics and non-asthmatics non users	**aOR = 1.79[q]**	**1.07, 2.99**
			1 month prior conception + 1st trimester	Salbut	25	887	101	6207	Asthmatics and non-asthmatics non users	**aOR = 1.65[r]**	**1.06, 2.58**
			1 month prior conception + 1st trimester	Salbut	15	1114	101	6207	Asthmatics and non-asthmatics non users	aOR = 0.76[s]	0.44, 1.33
			2nd & 3rd trimesters	Salbut	7	570	55	6207	Asthmatics and non-asthmatics non users	aOR = 1.34[q]	0.60, 2.98
			2nd & 3rd trimesters	Salbut	4	887	55	6207	Asthmatics and non-asthmatics non users	aOR = 0.52[r]	0.19, 1.44
			2nd & 3rd trimesters	Salbut	6	1114	55	6207	Asthmatics and non-asthmatics non users	aOR = 0.64[s]	0.27, 1.49
			1 month prior conception + 1st trimester	Pirbuterol/ Metaprot/ Epineph	4	85	5	153	Asthmatics and non asthmatics non users	cOR = 1.44	NA
Lin et al. (2009)	Matched Case Control 1:2	Registry, medical records & tel. interviews	1 month prior conception + 1st trimester	Salbut[g]	15	443	14	965	Asthmatics and non asthmatics with no Rx	aOR = 2.37[w]	0.90,6.23
				Metaprot[g]	1	31	1	42	Asthmatics and non asthmatics with no Rx	cRR = 1.35[w]	NA
				Terbut [g]	1	31	0	43	Asthmatics and non asthmatics with no Rx	—	NA
Lin et al. (2008)	Case Control 1:11	Tel. interviews & Rx DB	1 month prior conception + 1st trimester	Salbut/ Pirbuterol[g]	13	368	88	4,033	Asthmatics and non asthmatics non users [g]	cOR = 1.62[z]	NA
Tata et al. (2008)	Matched Case Control 1:6	THIN DB	Entire pregnancy	Any	375	NA	2085	NA	Asthmatics non users[bcd]	aOR = 1.06	(P = 0.336) 0.94,1.19
			1st trimester		NA	NA	NA	NA	NA	aOR = 1.01	(P = 0.941) 0.86,1.18

LABA only: cohort studies

Study ref.	Design	Source of data	Exposure Timing	Users of ß2-agonists			Non-users of ß2-agonists			Effect	
				Type of ß2-agonists	n*	Major congenital malformation (%)	Definition	n*	Major congenital malformation (%)	OR or RR	95% CI or (p-value)
Kallen (2014)	Cohort	Swedish Registers (Medical birth, Congenital malformation and Hospital discharge)	1st trimester (early pregnancy, usually 10-12 weeks)	Any	8947	3.20	General population	NA	NA	aOR = 1.08	0.96,1.22
				Any	8947	1.14	General population	NA	NA	aOR = 1.12[w]	0.92,1.36
Vasilakis-Scaramozza et al (2013)	Cohort	General Practice Research Database (GPRD)	180-335 days before LB, 70- 225 SB	Any	424	2.59	Non asthmatics non users	15840	2.78	cOR = 0.80	0.40,1.50
Eltonsy et al. (2011)	Cohort	Quebec administrative DB	1st trimester	Any	165	7.9	Asthmatics non users[g]	12,952	5.8	aOR = 1.31	0.74,2.31
						4.2			2.0	**aOR = 2.38[k]**	**1.11,5.1**
						1.8			0.5	**aOR =3.97[l]**	**1.29,12.2**
Kallen et al. (2007)	Cohort	Swedish Registry (Medical birth, Congenital malformation and Hospital discharge)	1st trimester	Salmeterol	NA	NA	General population	NA	NA	aOR = 1.34[m]	0.96,1.88
				Formoterol	NA	NA		NA	NA	aOR = 1.07[n]	0.63,1.82

LABA only: case-control studies

Study ref.	Design	Source of data	Exposure Timing	Type of ß2-agonists	Cases		Controls		Definition of non-users of ß2-agonists	Effect	
					Users of ß2-agonists	Non-users of ß2-agonists	Users of ß2-agonists	Non-users of ß2-agonists		OR or RR	(95% CI) or (p-value)
Lin et al. (2012)	Case Control	Registry, medical records & self reports	1 month prior conception + 1st trimester	Salmeterol	13	2840	23	6703	Asthmatics and non asthmatics non users	cOR = 1.33 [p]	NA
Munsie et al. (2011)	Case Control	Registry, medical records & self reports	1 month prior conception + 1st trimester	Salmeterol	6	83	21	137	Asthmatics and non asthmatics non users	cOR = 0.47[s]	NA
Lin et al. (2008)	Case Control 1:11	Tel. interviews & Rx DB	1 month prior conception + 1st trimester	Salmeterol[g]	2	379	11	4,110	Asthmatics and non asthmatics non users [g]	cOR = 1.97	NA
Tata et al. (2008)	Matched Case Control 1:6	THIN DB	Entire pregnancy	Any	25	NA	131	NA	Asthmatics non users[bcd]	aOR = 1.12	(P = 0.614) 0.72,1.75
			1st trimester		NA	NA	NA	NA		aOR = 1.09	(P = 0.77) 0.62,1.9

Table 2.6.B. Studies Investigating the Association between ICS Use during Pregnancy and Major Congenital Malformations

Study ref.	Design	Source of data	Exposure Timing	Users of ICS			Non-users of ICS			Effect	
				Type of ICS	n*	Major congenital malformation (%)	Definition	n*	Major congenital malformation (%)	OR or RR	95% CI or (p-value)
ICS use: cohort studies											
Charlton et al. (2015)	Cohort	General Practice Research Database (GPRD)	1st trimester +2 weeks before prior	Fluticasone	328 (moderate asthma)	2.44	Asthmatics users of other ICS	2598	2.31	aOR = 1.10	0.50,2.30
				Fluticasone	1274 (considerable to severe asthma)	2.67		1080	2.31	aOR = 1.20	0.70,2.00
Kallen (2014)	Cohort	Swedish Registers (Medical birth, Congenital malformation and Hospital discharge)	1st trimester (early pregnancy, usually 10-12 weeks)	Any	24594	3.32	General population	NA	NA	**aOR = 1.08**	**1.01,1.16**
				Any	24594	1.14	General population	NA	NA	aOR = 1.11[w]	0.99,1.25
Bjorn et al. (2014)	Cohort	Danish Medical Registries (Danish National Registry of Patients, Discharges and Prescriptions Database)	1 month prior conception + 1st trimester	Any	1223	4.30	Asthmatics and non-asthmatics non users	3446	4.30	cOR = 1.02	0.77,1.34
				Any	1223	0.08	Asthmatics and non-asthmatics non users	3446	0.20	cOR = 0.47[x]	0.07,3.34
Vasilakis-Scaramozza et al (2013)	Cohort	General Practice Research Database (GPRD)	180-335 days before LB, 70- 225 SB	Any	4735	3.17	Non asthmatics non users	15840	2.78	cOR = 1.10	0.90,1.40
Hviid et al. (2011)	Cohort	Danish Medical Birth Registry, National hospital Discharge Register & Danish Prescription Drug Register	1st trimester	Any	7421	0.81	Asthmatics and non-asthmatics non users	825215	1.05	aOR = 0.75[s]	0.34,1.68
				Any	7421	0.40	Asthmatics and non-asthmatics non users	825215	0.43	aOR = 0.94[r]	0.30,2.92
Blais et al. (2009)	Cohort	Quebec administrative DB	1st trimester	≥1000 mcg beclo equivalent	154	9.7	Asthmatics, users of ≥ 0-1000 mcg beclo	4392	5.7	aOR = 1.67	0.91,3.06

Blais et al. (2007)	Cohort	Quebec administrative DB	1st trimester	> 0–500 mcg beclo equivalent	1582	6.4	Asthmatics, non users of ICS	2740	6.0	aOR = 0.90	0.64,1.24
				> 500-1000 mcg beclo equivalent	167	3.6				aOR = 0.56	0.22, 1.43
				>1000 mcg beclo equivalent	72	9.7				aOR = 1.67	0.56, 5.03
Bakhireva et al. (2005)	Cohort	Tel. interviews & medical charts	Entire pregnancy	Any	438	4.10	Non asthmatics	303	0.30	cRR = 13.70	(P < 0.05)
				Any	438	4.10	Asthmatics ICS non users	103	3.90	cRR = 1.10	(P > 0.05)
Schatz et al. (2004)	Cohort + RCT	Medical charts & interviews	Entire pregnancy	Any	722	1.90	Asthmatics ICS non users	1401	2.00	cRR = 1.00	(P > 0.05)
Dombrowski et al. (2004)	RCT	Medical charts & interviews	Entire pregnancy	Beclo	193	3.10	Asthmatics theophylline users	189	2.60	cRR = 1.20	0.40, 3.80
Schatz et al. (1997)	Cohort	Daily diary cards for medications completed by patients, tel. interviews & medical charts	Entire pregnancy	Any[Y]	NA	5.40	Non asthmatics and ICS non users	NA	4.90	cRR = 1.10	(P >0.05)

ICS use: case-control studies

Study ref.	Design	Source of data	Exposure Timing	Type of ICS	Cases		Controls		Definition of non-users of ICS	Effect	
					Users of ICS	Non-users of ICS	Users of ICS	Non-users of ICS		OR or RR	95% CI or (p-value)
Garne et al. (2015)	Case-Malformed Control	EUROmediCAT database (Birth registry, medical records & self reports)	1st trimester	Any	133	16670	349[t]	43475	Asthmatics and non-asthmatics non users	aOR = 0.85	0.68, 1.07
				Any	133	16670	51[u]	9527	Asthmatics and non-asthmatics non users	aOR = 1.01	0.68, 1.49
Kallen et al. (2003)	Case control	Swedish Medical birth Register & Interviews	Early pregnancy	Any	66	7404	NA	577,730	Asthmatics and non asthmatics non users	aOR = 1.05	0.82, 1.34

Table 2.6.C. Studies Investigating the Association between LABA-ICS combination Use during Pregnancy and Major Congenital Malformations

Study ref.	Design	Source of data	Exposure Timing	Users of LABA-ICS			Non-users of LABA-ICS			Effect	
				Type of LABA-ICS	n*	Major congenital malformation (%)	Definition	n*	Major congenital malformation (%)	OR or RR	95% CI or (p-value)
LABA-ICS combination use: cohort studies											
Charlton et al. (2015)	Cohort	General Practice Research Database (GPRD)	1st trimester +2 weeks before prior	Fluticasone + salmeterol	177 (moderate asthma)	2.82	Asthmatic users of ICS other than fluticasone	2598	2.31	aOR = 1.30	0.50,3.20
				Fluticasone + salmeterol	1032 (considerable to severe asthma)	2.62		1080	2.31	aOR = 1.10	0.60,2.00
Kallen (2014)	Cohort	Swedish Registers (Medical birth, Congenital malformation and Hospital discharge)	1st trimester (early pregnancy, usually 10-12 weeks)	Any	8467	3.19	General population	NA	NA	aOR = 1.07	0.95,1.21
				Any	8467	1.02	General population	NA	NA	aOR = 1.01[w]	0.81,1.25

Study ref.	Design	Source of data	Exposure Timing	Type of LABA-ICS	Cases		Controls		Definition of non-users of LABA-ICS	Effect	
					Users of LABA-ICS	Non-users of LABA-ICS	Users of LABA-ICS	Non-users of LABA-ICS		OR or RR	95% CI or (p-value)
LABA-ICS combination use: case-control studies											
Garne et al. (2015)	Case-Malformed Control	EUROmediCAT database (Birth registry, medical records & self reports)	1st trimester	Any	60	16743	131[t]	43693	Asthmatics and non-asthmatics non users	aOR = 1.09	0.79, 1.49
				Any	60	16670	26[u]	9552	Asthmatics and non-asthmatics non users	aOR = 1.09	0.65, 1.83
				Any	3	645	131[t]	43693	Asthmatics and non-asthmatics non users	aOR = 1.69[o]	0.52, 5.48
				Any	3	645	26[u]	9552	Asthmatics and non-asthmatics non users	**aOR = 3.63[o]**	**1.26, 10.42**

Women participating in the different studies were asthmatic unless stated otherwise.
***** Number of pregnancies unless stated otherwise.
[a] Women may have concurrently received short-acting beta$_2$-agonists (inhaled or systemic).
[b] Women may have concurrently received inhaled corticosteroids.
[c] Women may have concurrently received asthma controller medications (leukotriene modifiers).
[d] Women may have concurrently received systemic corticosteroids (oral or intravenous).
[e] Women may have received inhaled, oral or injectable beta$_2$-agonists.
[f] Women may have concurrently received asthma controller medications (theophylline or ipratropium).
[g] Women may have concurrently received any other type of asthma medication.
[h] The OR presented for the association between salbutamol and cardiac malformations (92 cases reported)
[i] The OR presented for the association between terbutaline and cardiac malformations (228 cases reported)
[j] Women may have concurrently received asthma controller medications (cromolyn).
[k] The OR presented for the association between LABA and major cardiac malformations
[l] The OR presented for the association between LABA and major "other and unspecified malformations"
[m] The OR presented for the association between salmeterol and cardiac malformations (35 cases reported)
[n] The OR presented for the association between formoterol and cardiac malformations (14 cases reported)
[o] The OR presented for the risk of esophageal atresia
[p] The OR presented for the association between beta$_2$-agonists and selected defects including diaphragmatic hernia, esophageal atresia, small intestinal atresia, anorectal atresia, neural tube defects, omphalocele, or limb deficiencies with no additional major defect (isolated).
[q] The OR presented for cleft lip only
[r] The OR presented for cleft palate only
[s] The OR presented for cleft lip with cleft palate
[t] The OR presented using a control group with non-chromosomal malformations
[u] The OR presented using a control group with chromosomal malformations
[v] The OR presented for the association between bronchodilators use (mainly SABA) and anomalous pulmonary venous return (non-significant results for the other specific heart defects examined).
[w] The OR presented for major cardiac malformations
[x] The OR presented for oral clefts
[y] Women may have concurrently received intranasal corticosteroids.
[z] The OR presented for gastroschisis

DB: database; ICS: inhaled corticosteroids; Salbut: Salbutamol; Isoprot: Isoproterenol; Metaprot: Metaproterenol; Terbut: Terbutaline; Epineph: Epinephrine; Ephed: Ephedrine; Beclo: Beclomethasone dipropionate; SABA: Short acting beta$_2$-agonists; LABA: Long acting beta$_2$-agonists; RCT: randomized controlled trial; THIN: Health Improvement Network primary care database, Rx: prescription medications; aOR: adjusted odds ratio; cOR: crude odds ratio; cRR: crude risk ratio; cMD: crude mean difference; aMD: adjusted mean difference; pOR :crude prevalence odds ratio; NA: data unavailable; – : power or effect size impossible to calculate; NC: statistical power not calculated since results are significant. LB: live birth; SB: still birth.

2.7 Possible Teratogenic Mechanisms of Action for Beta$_2$-agonists and ICS

The biological mechanisms of teratogenicity of SABA, LABA and ICS are still uncertain, but several hypotheses exist. In regards to ICS, a proportion of the ICS that enter the systemic circulation may cross the placenta and affects the fetus, also diffusion of fluorinated corticosteroids (e.g. fluticasone and budesonide) is even more rapid than other corticosteroids.[145-147] Since fetal endogenous levels of corticosteroids are much lower than maternal levels, even minimal diffusions to the fetus could have a considerable impact.[148] Evidence shows that corticosteroids influences maternal hypothalamic-pituitary-adrenal (HPA) activity, which may play a role in endocrine and metabolic alterations in the offspring.[149] The early presence of the glucocorticoid receptor in the fetus implies that corticosteroids may affect the fetus by the glucocorticoid receptor and lead to persistent disorders in endocrine and metabolic control.[150] Animal models displayed potent teratogenicity of corticosteroids at doses less than or similar to those used in humans, with cleft palate being the primary malformation induced in most species.[146,151] Corticosteroids are essential for normal differentiation and growth of epithelial cells, but supraphysiologic doses interrupt this process.[152]

Animal and human data on selective SABA (e.g. salbutamol) have shown an acceptable safety profile.[153] However, non selective SABA like epinephrine can cause uterine vasoconstriction which could cause fetal harm.[147] SABA has rapid onset and short duration of action, which probably contributes to the absence of a human teratogenic effect that usually requires the exposure to a potential teratogen during a critical stage of the embryonic development that exceed a specific dose threshold. Salbutamol produces bronchodilation through stimulation of beta$_2$-adrenergic receptors in bronchial smooth muscle, thereby causing relaxation of bronchial muscle fibers.[154] Although beta$_2$-receptors are the predominant adrenergic receptors in bronchial smooth muscle and beta$_1$-receptors are the predominant receptors in the heart, there are also beta$_2$-receptors in the human heart comprising 10% to 50% of the total beta-adrenergic receptors.[154] The precise function of these receptors has yet to be established, raising the possibility that selective beta$_2$-agonists may also have cardiac effects. After inhalation, salbutamol plasma drug levels are very low.[154] However, it has been found that between 2% and 3% of salbutamol was transferred

from the maternal side to the fetal side of the placenta.[154] It is currently unknown if these minimal diffusions to the fetus have a potential teratogenic effect.

Regarding LABA, a probable teratogenic effect could arise from their potential effect on the corticosteroid function. Two different interactions of LABA on steroids have been identified, through which LABA could induce the gene transcription effect of steroids and subsequently their effects. First, LABA induce protein kinase A (PKA) activation which, in return, induces CAMP response element binding protein (CREB) binding protein (CBP). CBP activation is considered a rate limiting transcription factor for the steroids' action.[128] Second, LABA can directly induce ligand-independent nuclear translocation and activation of glucocorticoid receptors (GR) (i.e. induce migration of GR into the nucleus).[128,155] The theory postulates that by inducing the steroid-induced gene transactivation, LABA might also enhance the steroid-induced side effects[128] and among the possible side effects of oral corticosteroids maternal use is the increased risk of congenital malformations.[156-158]

2.8 Risk Factors for Congenital Malformations

2.8.1 Etiology of congenital malformations

It is now believed that causes of congenital malformations could be genetic, environmental, or unknown, including also interactions between those factors.[159-162] However, the specific etiology of most human major malformations is still unknown.[160,163] The genetic causes represent 15 to 25%, which include chromosomal abnormalities and new mutations.[160,162] Environmental causes, including maternal diseases, infectious agents, teratogenic drugs, alcohol, smoking and radiations, together represent about 10 to 15% of congenital malformations.[160,164] Finally, about 65 to 75 % are of unknown causes, where multifactorial gene-environment interactions as one of its main proportions, contributing about 20-25%.[159-162] In the following sub-sections and in Table 2.8 we will discuss some of the important risk factors for congenital malformations closely related to this thesis objectives. For their identification, we have reviewed the literature on asthma and asthma treatments during pregnancy, teratogenic medications and risks factors for major congenital malformations in order to determine relevant potential confounders.

2.8.2 Maternal characteristics, comorbidities and lifestyles

Maternal age is one of the major factors that affect pregnancy and perinatal outcomes, including major congenital malformations.[165-167] It has been shown that women at the extremes of the reproductive age distribution (< 18 years or ≥ 35 years) have an increased risk of congenital malformations compared to mid-age women.[165,166,168] Chromosomal anomalies, such as Down syndrome, is more prevalent among older women, but the risk for non-chromosomal anomalies is still equivocal.[165-167]

Among the environmental risk factors of congenital malformations is the area of residence. Urban or rural area of residence has been shown to be associated with significant changes in the prevalence of perinatal outcomes in several studies, including the prevalence of major malformations.[169-171] The urban versus rural residence status could be a major risk factor for some congenital malformations, reaching more than two folds increase in the prevalence of certain malformations among rural residents.[169-171]

Maternal education level and socioeconomic status were shown to be associated with several perinatal outcomes, including congenital malformations.[172-175] Compared to better-off women, women with low socioeconomic status had a higher risk of giving birth to a baby with a congenital malformation.[172,173,176] A study showed that having 10 years of schooling or less increases the risk of congenital malformations by almost three-folds compared to 4 years or more of higher education.[176]

An estimated 4% of the environmental causes of malformations is attributed to maternal conditions and maternal disease states[160], including chronic diseases like asthma, diabetes, chronic hypertension and epilepsy.[160,177,178] Good metabolic control in the preconceptional period was shown to be associated with decreased risk of congenital malformations.[179] According to several reports in the literature, pregnancies complicated by pre-existing maternal diabetes (both type-1 and type-2) have an approximately two to fourfold increased risk of major malformations.[180-182] Chronic hypertension affects about 3% to 5% of pregnancies, and its prevalence is increasing due to the rise in obesity and advanced maternal age.[65,183] Recently, more evidence became available on the effect of hypertension – separate from antihypertensive medications – on the prevalence of malformations. A recent study of over 800,000 pregnancies found that both treated and

untreated maternal chronic hypertension were associated with a 20-30% increase in the risk of major congenital malformations. [184] Similar results were observed for an increased risk of cardiac malformations.[184] Maternal overweight and obesity have shown associations with an increased prevalence of a variety of congenital malformations (e.g spina bifida, omphalocele and cardiac defects).[185-188] In a large meta-analysis, maternal obesity was found to be associated with an increased risk of pregnancies affected by neural tube defects (OR 1.87; 95% CI 1.62, 2.15), spina bifida (OR 2.24; 95% CI 1.86, 2.69), cardiovascular anomalies (OR 1.30; 95% CI 1.12, 1.51), septal anomalies (OR 1.20; 95% CI, 1.09, 1.31), cleft palate (OR 1.23; 95% CI, 1.03, 1.47), cleft lip and palate (OR 1.20; 95% CI, 1.03, 1.40), anorectal atresia (OR 1.48; 95% CI, 1.12, 1.97), hydrocephaly (OR 1.68; 95% CI, 1.19, 2.36), and limb reduction anomalies (OR 1.34; 95% CI, 1.03, 1.73).[185] Moreover, maternal obesity often leads to other morbidities (e.g. diabetes) which are themselves associated with increased prevalence of congenital malformations.[185-188]

Lifestyles and maternal habits include important risk factors for congenital malformations.[160] Maternal alcohol consumption can lead to a wide spectrum of birth defects, which range in frequency and severity from fetal alcohol-related defects to the distinctive fetal alcohol syndrome.[159,189,190] Maternal alcohol intake less than once per week was associated with a 1.6 to 2.1 fold increased risk of NTDs, d-transposition of the great arteries, and multiple cleft lip with or without cleft palate and more regular alcohol intake increased the risks for NTDs (OR 2.1, 95% CI: 1.1, 4.0) and cleft lip with or without cleft palate (OR 2.6, 95% CI: 1.1, 6.1).[191] While there is conclusive evidence that alcohol is teratogenic, there are no known levels of alcohol during pregnancy that is considered safe.[192] Fetal alcohol syndrome comes with significant costs in health, social, educational and other services of the society. In 2009, the estimated annual cost of fetal alcohol syndrome in Canada was $6.2 billion dollars.[193] Maternal smoking on the other side was examined in several studies to assess its association with congenital malformations.[189,190,194,195] In a large meta-analysis including over 173,000 malformed babies, tobacco smoking was associated with modest significant increases in digit anomalies, cryptorchidism and cardiovascular and musculoskeletal system anomalies (aORs 1.09–1.19); and larger significant increases (aORs 1.25–1.50) in limb reduction defects, clubfoot, oral clefts and defects of the eyes and gastrointestinal system (gastroschisis and abdominal hernias).[194]

Other maternal conditions and maternal diseases were reported to have a teratogenic effect on the fetus. The list include: Cushings disease (increased risk of hyperadrenocortism), iodine deficiency (causing embryonic goiter and mental retardation), maternal androgen endocrinopathy (embryonic masculinization), reduced maternal folic acid intake (increased incidence of NTDs), maternal phenylketonuria (untreated maternal phenylketonuria is associated with a 6-fold-increased risk of heart defects), maternal starvation (increased risk of NTDs) and Zinc deficiency: (possible increased risk of NTDs).[147,160,163,164,196-198]

Maternal exposure to toxins, chemicals and pollutants have been examined in previous reports, with several potential environmental teratogens being identified. The list include: carbon monoxide poisoning (CNS damage has been reported with very high exposures), lead (very high exposures can cause pregnancy loss and intrauterine teratogenesis), gasoline addiction embryopathy (increased risk of facial dysmorphology and mental retardation), methyl mercury (Minamata disease [cerebral palsy, microcephaly, mental retardation, blindness, cerebellum hypoplasia]), polychlorinated biphenyls (increased risk of CNS malformations, Cola-colored babies, pigmentation of gums, nails, teeth and groin; hypoplastic deformed nails; intrauterine growth retardation; abnormal skull calcification) and toluene addiction embryopathy (facial dysmorphology and mental retardation).[147,159,163,197-200]

2.8.3 Fetal conditions and infections

Embryonic and fetal infections contribute about 1% to 3% of the malformations in humans.[159,160] Embryonic and fetal infections that have a proven teratogenic effect includes cytomegalovirus, herpes simplex virus, lymphocytic choriomeningitis virus, rubella virus, toxoplasmosis, syphilis, and varicella-zoster.[72,160,189] These infections and their resulting syndromes are typically referred to as TORCH (Toxoplasrna gondii, Other microorganisms including syphilis, Rubella virus, Cytomegalovirus, and herpes viruses).[201,202] While they often produce mild maternal morbidities, they cause serious fetal consequences in some cases, including fetal death. Common malformations attributed to TORCH infections include cardiac defects, occular lesions, hearing defects, central nervous system defects, neonatal purpuras, and hepatosplenomegaly.[201-203] Congenital toxoplasmosis has a wide spectrum of clinical manifestations, with 10% of affected newborns suffering from systemic congenital defects.[201] The majority of infants with congenital cytomegalovirus

infection have no apparent clinical manifestations, but approximately 5% to 15% of the children suffer major abnormalities/defects such as hearing loss, microcephaly, mental retardation, and motor defects.[201,203] Maternal infection with rubella early during pregnancy was associated with a 70% increased risk of congenital heart lesions.[203]

Recently, Zika virus (ZIKV) – an emerging mosquito-borne flavivirus – has attracted a global attention.[204] The ZIKV infection has increased dramatically in 2015 throughout the Americas, with Brazil being the most affected country. The preliminary estimates in Brazil reached 440,000 to 1.3 million cases of autochthonous ZIKV infection reported through December 2015.[205,206] The report from the Ministry of Health of Brazil suggest that cases of microcephaly have increased by a factor of approximately 20 among newborns in the northeast region of the country.[207] The World Health Organization (WHO) has declared the clusters of microcephaly and other neurological disorders to be a Public Health Emergency of International Concern.[208] Beside microcephaly, the potential adverse outcomes in babies whose mothers were infected during pregnancy include also incomplete brain development and Guillain-Barré syndrome (GBS).[204,205,209,210] As of September 22, 2016, 282 travel-related cases, 2 sexually transmitted cases and 2 reports of maternal-to-fetal transmission have been detected in Canada. To date, Public Health Canada has confirmed two maternal-to-fetal transmissions of Zika virus, including one with severe neurological congenital anomalies (http://healthycanadians.gc.ca/diseases-conditions-maladies-affections/disease-maladie/zika-virus/surveillance-eng.php#s1).

2.8.4 Pregnancy related characteristics

Several studies have reported an increased prevalence of congenital malformations among multiple births compared to singletons.[211-213]. In a study including 27,727 multiple births and 944,967 singletons, multiple births was associated with a significant increased risk of major congenital malformations (OR 1.46, 95% CI: 1.42, 1.50).[212] In two large studies, significant increased risks were found for several system specific categories of malformations including anencephalus, biliary atresia, hydrocephalus without spina bifida, pulmonary valve atresia and stenosis, bladder exstrophy, macrocephaly, encephalocele, cleft lip and palate, anomalies of the diaphragm, cardiac septal defects, atresia or stenosis of the large intestine or anus, tracheoesophageal fistula, malformations of the alimentary

tract, inguinal and umbilical hernias, and cystic kidney, with estimated odds ratios ranging between 1.24 and 7.44.[211,212]

2.8.5 Asthma related variables

The total body of published evidence show that maternal asthma could significantly increase the risk of major congenital malformations.[2,5,7-9] Other related factors include the asthma severity and control levels and the asthma medications used to control its symptoms. Oral corticosteroids use during the first trimester was associated with an increased prevalence of congenital malformations in previous reports, especially orofacial clefts.[34,131,214] For example, in a large study by the National Birth Defects Prevention Study (NBDPS) Group, maternal use of oral corticosteroids was associated with a significant increased risk of cleft palate (aOR 1.7, 95% CI, 1.1, 2.6).[215] As previously discussed, severe and uncontrolled asthma are potential risk factors for congenital malformations and should be adjusted for properly.[14,43,98,216] Numerous studies have shown associations between suboptimal control of asthma and more severe asthma during pregnancy and increased maternal and fetal risks.[2,5,7-9] In contrast, better-controlled asthma and mild-to-moderate actively managed asthma are associated with decreased risks.[10,11]Asthma severity and control can be assessed through several methods (details in Chapter 2, section 2.3). Among the key markers of asthma severity and control that can be used are the emergency department (ED) visits for asthma, hospital admissions for asthma, the use of oral corticosteroids and the SABA doses used per week.[121,144,217,218]

2.8.6 Teratogenic medications use during pregnancy

The term teratogen stands for an agent that can produce structural or functional abnormalities in an exposed embryo or fetus.[189,219] Prescription and over the counter (OTC) medications are part of the environmental causes of congenital malformations, contributing around 1%.[159] Congenital malformations attributed to their use certainly have a special importance, since they could be preventable. [72,159,189] Disagreements arise when trying to establish the specific criteria to identify and label medications as teratogens, nonetheless; the dose, route of exposure and gestational timing of the exposure play the major role in identifying any teratogen.[160,219,220]

Most women take medications at some point during pregnancy, either for the treatment of acute illnesses (e.g. heartburn and nausea) or for management of chronic diseases predating or accompanying gestation (e.g. asthma, epilepsy and depression).[221-224] A large proportion of maternal medication use during pregnancy involves OTC medications, but there is also considerable use of prescription medications – prescription and OTC medications use estimates ranged between 27% and 99%, based on the medications examined and the data sources used.[221-223]

Evidence of proven teratogenic effect has been established for a number of currently available medications, acting through various mechanisms, including folate antagonism, vascular disruption and oxidative stress.[17] However, the majority of medications lack sufficient data to appropriately evaluate their teratogenicity in humans.[222,225] A Dutch drug utilization study found that 17.5% of women in the examined cohort have received one or more prescription drugs suspected to be associated with a teratogenic mechanism during the first trimester of pregnancy [224], and in the United States, a study showed that 23% of the medications most commonly used during the first trimester were included in Category X of the U.S. Food and Drug Administration classification (risks involved in use of the drug clearly outweigh potential benefits.).[225]

In a study published in 2011 based on expert reviews by the Teratology Information System (TERIS), the authors found that among 172 medications approved in the United States between 2000 and 2010, 97.7% had insufficient published data and 73.3% had no human data with which to determine their teratogenic risk in humans.[164] Typically, when medications become available for longer periods of time in the markets and increasingly used by pregnant women, more evidence accumulate and a growing number of medications become eventually recognized as teratogens based on solid established human data (e.g. mycophenolate mofetil). Therefore, new evidence is constantly produced for currently marketed medications, and several information sources can be accessed for the assessment of their teratogenic risk. [62,63,67,71-73,226-233] Those information sources – usually online databases and reference books – on teratogenic risks provide complete or partial evidence for the teratogenicity of medications.[62-73,233] However, there are substantial discrepancies between the lists of medications that should be considered teratogenic, and significant imprecision is added when categories are used (e.g., moderate- vs high-risk teratogens).[62-73] Moreover, currently available lists are outdated at some levels.[64,67-70]

In observational studies of congenital malformations, it is essential to control for the maternal exposure to proven and potential teratogenic medications, as failure to do so can affect the study validity. As mentioned previously, the increase in the body of knowledge on currently used medications make it difficult to identify a fixed list of teratogenic medications that should be used in research. Besides, the task becomes even more difficult when the potential teratogenic medications or medications with evidence of small teratogenic risk are being considered.[234] For that reason, several researchers have developed their own teratogenic or potential teratogenic medications lists that they use in their own research. Such lists usually become problematic as they are inherently subjective in nature, especially when potential teratogens are included, and they require constant review of the literature to incorporate the new evidence and updates.

To demonstrate such problematic issue, we carefully examined the studies that controlled for the maternal exposure to teratogenic medications presented in Table 2.8 below.[29,30,34,49] Two studies used a teratogens list that included only proven teratogens with 17 medications and 7 medication classes that was published in 1998.[34,49] One study used a list including only 12 medications[29] and one study used a longer list of over 150 medications that included both teratogenic and potential teratogenic medications.[30] Examining the literature in fields other than asthma during pregnancy, we often find similar discrepancies. A recent study on the effect of topiramate use during pregnancy on the prevalence of oral clefts used a list of proven and suspected teratogenic medications that contained 39 medications and 6 medication classes.[235] However, the list included all statins but excluded other important teratogens (e.g. mycophenolate mofetil).[235] The examples mentioned above highlight the importance of having an updated and thoroughly examined – yet objectively developed – teratogens list to be used in congenital malformations research.

2.8.7 Potential confounders

From the published literature on major congenital malformations risk and maternal asthma medications use reviewed earlier in section 2.6, we congregated the several risk factors considered in the studies, which we present in Table 2.8. As shown in the table, some risk factors are considered of major importance and were considered as potential

confounders in nearly all studies (e.g. maternal age), while others were rarely controlled for due to their questionable importance (e.g. caffeine use). Notably, the asthma related variables are considered some of the key potential confounders that affect the studies' validity.

Through examining the different risk factors, we identified some variables that will be impossible to capture through the databases used in our current research, including for example maternal smoking, folic acid use and BMI. However, the databases will allow us to adjust for important potential confounders like socioeconomic status and fetal infections. Typically, a risk factor has to be associated with the exposure variable in the study for it to be considered as a potential confounder, which is in our case the choice of the treatment regimen being a combination therapy or monotherapy.[236] The potential confounders that we can measure in our databases and will be considered in our statistical analysis include: maternal age at the beginning of pregnancy, receipt of social assistance during pregnancy, area of residence at delivery, chronic hypertension, diabetes mellitus, exacerbation of asthma (defined as a filled prescription for OCS, an emergency department visit, or a hospitalization for asthma) three months before pregnancy, and SABA dose per week in the three months preceding pregnancy.

Table 2.8 Risk factors considered as potential confounders in the literature review

Risk factor	Studies including the risk factor	Included in the current studies
Maternal characteristics, lifestyle habits and comorbidities		
Maternal age	Bakhireva et al. (2005), Schatz et al. (2004), Garne et al. (2015), Van Zutphen et al. (2015), Lin et al. (2012), Munsie et al. (2011), Lin et al. (2009), Lin et al. (2008), Kallen (2014), Vasilakis-Scaramozza et al (2013), Eltonsy et al. (2011), Kallen et al. (2007), Schatz et al. (1997), Tata et al. (2008), Charlton et al. (2015), Bjorn et al. (2014), Hviid et al. (2011), Blais et al. (2009), Blais et al. (2007), Kallen et al. (2003)	Yes

Socioeconomic status	Bakhireva et al. (2005), Van Zutphen et al. (2015), Eltonsy et al. (2011), Tata et al. (2008), Charlton et al. (2015), Hviid et al. (2011), Blais et al. (2009), Blais et al. (2007)	Yes
Insurance status (public vs private)	Schatz et al. (2004)	No
Area of residence	Eltonsy et al. (2011), Hviid et al. (2011), Blais et al. (2009), Blais et al. (2007)	Yes
Race/ ethnicity	Bakhireva et al. (2005), Schatz et al. (2004), Van Zutphen et al. (2015), Lin et al. (2012), Munsie et al. (2011), Lin et al. (2009), Lin et al. (2008), Schatz et al. (1997)	No
Education level	Van Zutphen et al. (2015), Lin et al. (2012), Munsie et al. (2011), Lin et al. (2008), Eltonsy et al. (2011), Kallen et al. (2007), Hviid et al. (2011), Blais et al. (2009), Blais et al. (2007)	No
Maternal country of birth	Kallen et al. (2007), Hviid et al. (2011)	No
Weight/ body mass index	Bakhireva et al. (2005), Van Zutphen et al. (2015), Lin et al. (2012), Munsie et al. (2011), Lin et al. (2009), Kallen (2014), Vasilakis-Scaramozza et al (2013), Kallen et al. (2007), Schatz et al. (1997), Tata et al. (2008), Charlton et al. (2015)	No
Smoking status	Bakhireva et al. (2005), Schatz et al. (2004), Van Zutphen et al. (2015), Lin et al. (2012), Munsie et al. (2011), Lin et al. (2008), Kallen (2014), Vasilakis-Scaramozza et al (2013), Kallen et al. (2007), Schatz et al. (1997), Tata et al. (2008), Charlton et al. (2015), Bjorn et al. (2014), Hviid et al. (2011), Blais et al. (2007), Kallen et al. (2003)	No
Alcohol consumption	Bakhireva et al. (2005), Van Zutphen et al. (2015), Lin et al. (2012), Munsie et al. (2011), Charlton et al. (2015), Blais et al. (2007)	No
Illicit drug use	Lin et al. (2012), Munsie et al. (2011), Blais et al. (2007)	No
Caffeine use	Lin et al. (2009)	
Hypertension	Eltonsy et al. (2011), Blais et al. (2009)	Yes

Diabetes	Bakhireva et al. (2005), Lin et al. (2009), Vasilakis-Scaramozza et al (2013), Eltonsy et al. (2011), Bjorn et al. (2014), Hviid et al. (2011), Blais et al. (2009), Blais et al. (2007)	Yes
Maternal epilepsy	Eltonsy et al. (2011), Hviid et al. (2011), Blais et al. (2009), Blais et al. (2007)	Yes
Thyroid disorder	Blais et al. (2007)	No
Family history of congenital malformations	Lin et al. (2009), Hviid et al. (2011), Blais et al. (2007)	No
History of infertility	Vasilakis-Scaramozza et al (2013), Kallen et al. (2007), Kallen et al. (2003)	No
Maternal history of miscarriage	Kallen et al. (2007), Blais et al. (2007)	No
Pregnancy related characteristics		
Year of delivery	Kallen (2014), Kallen et al. (2007), Hviid et al. (2011), Kallen et al. (2003)	No
Multiple pregnancy	Eltonsy et al. (2011), Blais et al. (2009), Blais et al. (2007)	Yes
Parity	Bakhireva et al. (2005), Schatz et al. (2004), Lin et al. (2012), Munsie et al. (2011), Kallen (2014), Eltonsy et al. (2011), Kallen et al. (2007), Schatz et al. (1997), Hviid et al. (2011), Blais et al. (2009), Blais et al. (2007), Kallen et al. (2003)	No
Gravidity	Bakhireva et al. (2005)	No
Preterm delivery	Vasilakis-Scaramozza et al (2013), Tata et al. (2008)	No
Fetal infections	Eltonsy et al. (2011), Hviid et al. (2011)	No
Teratogenic medication use	Vasilakis-Scaramozza et al (2013), Eltonsy et al. (2011), Blais et al. (2009), Blais et al. (2007)	Yes
Folic acid use	Van Zutphen et al. (2015), Lin et al. (2012), Munsie et al. (2011), Lin et al. (2008), Blais et al. (2007)	No
Vitamin use	Lin et al. (2009), Blais et al. (2007)	No
Vasoactive medications use	Lin et al. (2008), Lin et al. (2008)	No
Exposure to irradiation or x-rays	Blais et al. (2007)	No
Infant sex	Van Zutphen et al. (2015), Lin et al. (2012), Munsie et al. (2011), Lin et al. (2009), Tata et al. (2008)	No

Fever during the first trimester	Munsie et al. (2011), Lin et al. (2009)	No
Trihalomethane exposure	Lin et al. (2009)	No
Uterine complications (including uterine defects and amniotic bands)	Eltonsy et al. (2011)	No
Use of benzodiazepines, analgesics, beta-blockers and oral contraceptives	Hviid et al. (2011)	No
Asthma related variables		
ICS maternal use	Garne et al. (2015), Eltonsy et al. (2011), Blais et al. (2007)	Yes
SABA use	Garne et al. (2015), Blais et al. (2009), Blais et al. (2007)	Yes
LABA use	Garne et al. (2015), Blais et al. (2009), Blais et al. (2007)	Yes
Other asthma controller medications use	Eltonsy et al. (2011), Blais et al. (2009)	Yes
Intranasal corticosteroids use	Eltonsy et al. (2011), Blais et al. (2009), Blais et al. (2007)	No
Oral corticosteroids use	Eltonsy et al. (2011), Charlton et al. (2015), Blais et al. (2009), Blais et al. (2007)	Yes
Emergency department (ED) visit or hospitalization for asthma	Schatz et al. (2004), Eltonsy et al. (2011), Blais et al. (2009), Blais et al. (2007)	Yes
Exacerbations/acute asthma attacks	Schatz et al. (1997), Charlton et al. (2015)	Yes
Asthma severity level	Bakhireva et al. (2005), Eltonsy et al. (2011), Charlton et al. (2015), Blais et al. (2009)	No
Asthma control	Eltonsy et al. (2011), Charlton et al. (2015), Blais et al. (2009)	No
FEV_1	Schatz et al. (2004), Schatz et al. (1997)	No

2.9 Case ascertainment definitions of major congenital malformations

Accurate identification of major congenital malformations from administrative databases is a key requirement for reaching valid results in studies based on cohorts selected from those administrative databases. Computerized health administrative databases have become an important source of data for congenital malformations research. Multiple sources of data have been used in prior studies that assessed cases of congenital malformations, including hospital discharge data, vital records, specialty clinic data, and billing claims data.[53,237] The recent reports from the Public Health Agency of Canada showed that major congenital malformations are present in approximately 3%–5% of newborns and 8%–10% of stillbirths in Canada.[16] However, several published studies have demonstrated reasons which lead to discrepancies in the estimated prevalence of major malformations. [53-55,237-240] These reasons include the case ascertainment method (e.g. active [by trained abstractors], passive [through unverified direct reporting], or a combination of both), the source of data, the validity of the diagnostic codes, the classification method into minor or major malformations, and the period of assessment (e.g. at birth or during the 1st year of life).[53-55]

Both the RAMQ – Quebec's Medical Claims database – and the MED-ECHO – Quebec's hospital discharge summary database – have been used for congenital malformations research.[56,58,59,241] Of note, the national Canadian Congenital Anomalies Surveillance System (CCASS) and the Quebec's Minister of Health and Social Services both rely on the MED-ECHO database for reporting the prevalence of congenital malformations in Quebec.[16,242] Two recent validation studies examined the accuracy of the congenital malformations diagnoses recorded in MED-ECHO and RAMQ databases.[60,243] Using data from RAMQ, MED-ECHO and the Births and Deaths Registry, Kulaga et al. examined the agreement between the congenital malformations recorded in these databases and those in the maternal reports from a self-administered questionnaire. A proportion of agreement of 60% was found between the database records and the mothers' reports, and among those who were concordant, the mother reported the same diagnosis as recorded in the databases in 90% of the cases.[60] In a recently published validation study, Blais et al. examined the validity of congenital malformations diagnostic codes among asthmatics and non-asthmatics recorded in the RAMQ, MED-ECHO and the Births and Deaths Registry using the infants' medical charts recorded by the physicians as the gold standard.[243] The

PPV for the outcomes any congenital malformations and major congenital malformations were 82.2% and 78.1% respectively among asthmatic, and 79.2% and 69.0% respectively among non-asthmatic women.[243] In another validation study, the validity of MED-ECHO in the identification of neural tube defects (NTD) was tested against hospital medical charts and death and stillbirth certificates.[59] Compared to the total number of NTD in all data sources, MED-ECHO had a high sensitivity (92%), but its PPV for the NTD ICD-9 codes 740.0 to 742.0 was only 56%.[59] Similar results were reported using comparable health administrative databases.[244-246] In a validation study by Devine et al using the general practice research database (GPRD) to identify children with neural tube defects, the overall reported PPV was 71% (95% CI = 63 to 78%). However, the PPV varied considerably with the specific NTD diagnosis.[244] Concerns over false positive cases of NTD among live births in MED-ECHO files was raised, where an NTD code can be recorded for an infant with a suspicion of NTD, even if the diagnosis was not formally confirmed during hospitalization.[58] To date, however, no study has examined the impact of the source of data on the estimated prevalence of congenital malformations using the MED-ECHO and/or RAMQ databases.

In fact, it is currently unknown how the congenital malformation diagnoses recorded in the Medical Claims database (i.e. RAMQ) would affect the estimated total prevalence, aside from MED-ECHO estimates. Some reports using comparable administrative databases and surveillance systems in other Canadian provinces and the United States were published.[54,55,238,239,247,248] For example, Bedard et al. used the Alberta Congenital Anomalies Surveillance System (ACASS), which links hospital, vital statistics, and medical genetic departments databases. They reported a prevalence of congenital heart defects of 5.59 per 1000 births, which increased to 12.42 per 1000 births when they added data from the outpatient pediatric cardiology clinic database and the hospital records for terminations of pregnancy.[55] However, their active review of health records (involving manual searches for cases) and the duration of follow-up (up to 15 years after delivery) might have influenced the results.[55] Metcalfe et al., who used the ACASS database as the gold standard, reported an accurate identification rate of 86.9% for congenital malformations recorded in the hospitalization database versus 51.1% in an outpatient visits database.[248] The PPV decreased when several databases were used to identify congenital malformations, which indicates that false-positive cases were included in the results.[248]

However, the authors did not confirm the cases using medical records, and the true percentage of false-positive cases was unknown. In a published validation study using the Tennessee births and mothers linked data, the PPV of all malformations detected through inpatient claims compared to medical records was 69.9%, with PPV values varying considerably by the organ system involved (e.g. 48.9% for central nervous system, 74.5% for cardiac and 93.3% for orofacial malformations).[239] The source of data used for cases ascertainment - being a registry, an active surveillance database, an administrative claims database or another type of database - has been identified as one of the potential sources of variability in the reported prevalence of congenital malformations.[53] In prior reports, 5%–20% of cases of major congenital malformations were false positives, and results vary according to the malformation categories and are rarely generalizable between data sources and classification methods.[55,238,248,249] Beside the variation in the prevalence of congenital malformations among studies, the case ascertainment definitions might also influence the estimates for the associations between maternal exposures and congenital malformations. Through our literature search, we could not locate a study that examined this objective specifically.

2.10 Knowledge gaps to be addressed

This thesis is partitioned into 3 parts, presented by 4 articles. The first part includes a systematic review, the second part includes one comparative safety study and one methodological study, and the third part includes an evidence-synthesis study.

As presented in the literature review above, a large body of evidence exists for SABA use during pregnancy, presented in several published articles.[2,26,30,102] The case is different for LABA where small evidence exists and few published studies can be retrieved.[30,96,129] Some systematic reviews exist on the use of asthma controller medications during pregnancy and maternal and fetal outcomes, however none examined SABA and LABA specifically. Moreover, the methodological limitations of the published studies and their statistical power merit a critical examination in a well-designed systematic review. In order to validly assess the perinatal safety profile of SABA and LABA use during pregnancy, a large systematic review including several important perinatal outcomes is needed.

Examining the fetal safety of asthma treatment regimens is highly important and has been the objective of several published articles. Yet, confounding by indication and the severity of asthma itself frequently obscured the results of previous studies, prohibiting valid inference on the fetal safety of important treatment regimens. Among the commonly prescribed treatment regimens used to manage asthma among pregnant women are ICS monotherapy and LABA-ICS combination therapies. Both treatment regimens are used among pregnant women with moderate to severe persistent asthma. Among the important decisions that physicians must make if asthma cannot be controlled with a low dose of ICS is whether to prescribe a LABA to supplement the current dose of ICS or to increase the dose of ICS. However, there has been no direct comparison of these treatment regimens to guide physicians on which treatment regimen is safer for the newborn. In the first article of the second part of this thesis, we will tackle this clinically important question by conducting the first comparative safety study examining the prevalence of major malformations of these two widely used treatment options for persistent asthma during pregnancy, namely LABA-ICS combination therapy versus ICS monotherapy at higher doses.

The second article of the second part of this thesis will be a methodological study investigating the case ascertainment methods of major congenital malformations in the RAMQ and MED-ECHO databases. The previously published validation studies did not examine the difference in the prevalence of major malformations using different case ascertainment definitions that vary by the source of data (i.e. when the RAMQ billing claims are added or not to the MED-ECHO hospitalizations diagnoses). Due to the increasing use of both databases in perinatal epidemiology, the examination of different case ascertainment definitions to be used in research is warranted. Moreover, examining the impact of different case ascertainment definitions – that vary by the source of data and the classification method – on a maternal exposure-major malformations association is of high relevance for applicability to future research.

The third part of this thesis will cover our approach to tackle the issue of the discrepancies and inconsistencies of teratogenic medications lists that can be used in research. Using incomplete or inaccurate lists in research represent an evident validity threat. The teratogens lists provided in earlier reports lack a systematic procedure for the classification of medications, even with the availability of relevant references and peer-

reviewed citations. The literature lacks the presence of proven and potential teratogens lists to be used in research that are both systematically and subjectively developed. Despite the presence of several reliable resources on teratogenic risks, the currently available lists are outdated on several levels and there is no consensus among researchers on the preferred lists to use. For that reason, harnessing the full potential of several reliable resources is essential to the creation of a comprehensive overview. Therefore, based on the currently available leading teratology resources, we planned to develop a systematic and updatable procedure for the classification of medications into proven and potential teratogens during the first trimester of pregnancy for use in research.

CHAPTER 3: OBJECTIVES

Objectives of the research program presented in the thesis

General objective of the thesis:

We sought to examine the comparative safety of two common treatment regimens for maternal asthma during the first trimester of pregnancy, as well as to solve some of the methodologic questions that could add important knowledge in this field.

The hypotheses and objectives of the four articles enclosed in the current thesis are listed below.

3.1 Systematic review on beta$_2$-agonists and perinatal outcomes

We hypothesized that the maternal use of beta$_2$-agonists during pregnancy could possibly be associated with an increased risk of several adverse perinatal outcomes.

3.1.1 Primary objective

To summarize the existing human data examining the impact of the use of inhaled SABA and LABA for the treatment of asthma during pregnancy on several perinatal outcomes, namely major and any congenital malformations, small for gestational age (SGA), birth weight, low birth weight (LBW), gestational age and preterm delivery.

3.1.2 Secondary objective

To assess the quality of each study using a validated quality assessment scale and perform post-hoc power calculations to evaluate the capacity of the studies in detecting clinically significant effects.

3.2 LABA-ICS combination therapy versus ICS monotherapy and major congenital malformations

We hypothesized that the risk of major congenital malformations in pregnant asthmatic women treated with LABA and ICS combination is higher than those treated with a higher dose of ICS monotherapy.

3.2.1 Primary objective

To compare the prevalence of major congenital malformations in pregnant asthmatic women treated with a combination of LABA and ICS and those treated with a higher dose of ICS monotherapy.

3.3 Case ascertainment definitions of major congenital malformations

We hypothesized that using different case ascertainment definitions – that vary by the source of data and the classification method – will affect the observed prevalence of major congenital malformations and influence the association between the maternal exposure and major congenital malformations.

3.3.1 Primary objective

To compare the prevalence of major congenital malformations using different case ascertainment definitions that vary by the source of data and the classification method.

3.3.2 Secondary objective

To evaluate the impact of these case ascertainment definitions on the association between maternal asthma and major congenital malformations.

3.4 Systematic Procedure for the Classification of Proven and Potential Teratogens

We hypothesized that the currently available lists of proven and potential teratogens used for research are outdated, and an updatable and systematic procedure could better identify and classify medications into proven and potential teratogens.

3.4.1 Primary objective

To develop a systematic and updatable procedure for the classification of medications into those with sufficient evidence of teratogenic risk and those with potential teratogenic risk during the first trimester of pregnancy for use in research.

CHAPTER 4: METHODS

Methods

This chapter covers the methods presented in the four manuscripts included in Chapter 5 more comprehensively, and includes a description of analyses that were not reported in the manuscripts. The first part of this chapter will cover the systematic review article and the details absent from its manuscript. The second part of this chapter will cover the sources of data used in the two manuscripts on the comparative safety of asthma treatment regimens and the case ascertainment definitions of congenital malformations. The third part will include details on the exposure assessments, outcome definitions and potential confounders present in those two manuscripts. The fourth part will cover some additional details on the teratogenic and potential teratogenic medications project.

4.1 Systematic review on beta$_2$-agonists and perinatal outcomes

This section covers the methodologic details that were not reported in the published systematic review manuscript presented in Chapter 5 due to space limitations.

4.1.1 Data sources and search strategy

We searched six databases for original articles: PubMed, Ovid MEDLINE, EMBASE, Cochrane Library, Web of Science, and CINAHL. Prior to commencing the search, a systematic review protocol was formed, registered and published in PROSPERO, the International prospective register of systematic reviews; cited as PROSPERO 2011:CRD42011001554, (Full details presented in Appendix A, also available at: http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42011001554).[250] Changes that occurred since the protocol publication includes the addition of the quality assessment of the included studies using a recognized scale. The quality assessment scale chosen was the Newcastle-Ottawa Scale (NOS) for the quality assessment of nonrandomized studies (details presented below).

4.1.2 Data extraction and study selection

We chose seven outcomes that best represent the fetal development (major and any malformations, SGA, mean and low birth weight) and the newborn prematurity (gestational

age and preterm delivery) among asthmatic women treated with SABA and LABA (full definitions in the manuscript). The full search strategy, including the inclusion and exclusion criteria, is presented in the manuscript in Chapter 5.

4.1.3 Quality assessment

The quality assessment of the included studies was performed using the Newcastle-Ottawa Scale. A 'star system' is used in which a study is judged on three broad perspectives: the selection of the study groups; the comparability of the groups; and the ascertainment of either the exposure or outcome of interest for case-control or cohort studies respectively.[251] The NOS and the manuals for both cohort and case-control studies are presented in Appendix B.

4.1.4 Reporting methodology

In order to ensure effective and precise reporting of the results from the conducted systematic review, we used a recognized reporting guidelines: the PRISMA statement.[252] PRISMA stands for Preferred Reporting Items for Systematic Reviews and Meta-Analyses.[252] It is an evidence-based minimum set of items for reporting in systematic reviews and meta-analyses. The aim of the PRISMA Statement is to help authors improve the reporting of systematic reviews and meta-analyses, through ensuring clear presentation of what was planned, done, and found in the systematic review and meta-analyses.[252] The PRISMA statement consists of a 27-item checklist and a 4-phase flow diagram.[252] The flow diagram results were included in the manuscript in Chapter 5 and the 27-item checklist is presented in Appendix C.

4.2 Sources of data

This subsection will cover the sources of data used in two of the four manuscripts in this thesis; the article on the comparative safety of asthma treatment regimens (LABA-ICS combination versus ICS monotherapy in higher doses) and the article on the case ascertainment definitions of major congenital malformations.

For both articles, we used the *Quebec Asthma and Pregnancy Database,* which links pregnancy data from two health administrative databases in Quebec: the Régie de l'assurance maladie du Québec (RAMQ) and the Maintenance et exploitation des données pour l'étude de la clientèle hospitalière (MED-ECHO) databases. Both databases – the RAMQ and MED-ECHO – have been used before by the research team of Dr. Lucie Blais in several studies in the field of asthma and perinatal outcomes.[30,34,121,127,253,254] They also have been frequently used by several researchers in different domains, including pregnancy outcomes research, cardiovascular diseases, infections, among several others.[57,137,255-257]

4.2.1 Régie de l'assurance maladie du Québec (RAMQ)

The RAMQ is responsible for the health insurance coverage of 7,9 million individuals in Quebec, including 3,5 million covered with the RAMQ Public Drug Insurance Plan.[258] Since 1997, all Quebec residents who were not covered by a private drug insurance plan were required to register with the RAMQ Public Drug Insurance Plan. By 2015, among the 3,5 million covered by the RAMQ Public Drug Insurance Plan there were 51% adherents (under 65 years of age and not covered by private insurance at their workplace), 35% elderly (over 65 years) and 14% recipients of social assistance.[258]

The RAMQ database provides the information on medical services dispensed to all residents. The RAMQ is the Quebec claims database for medical services provided paid on a fee-for-service bases. The RAMQ database used to construct *Quebec Asthma and Pregnancy Database* come in multiple files that were linked together using a unique identifier for each individual, i.e. the health insurance number. The sociodemographic information on the insured individuals include sex, place of residence (3 digits' postal code), date of birth and death (if deceased), among others. Information on the admissibility to the Public Drug Insurance Plan include the date of the beginning and end of the admissibility, among others.[258]

The medical services file contains – among others – data on each medical service provided, records the date the service is dispensed, where it is dispensed (clinic, emergency department, hospital), a diagnosis coded with ICD-9 codes, a procedure code, and the specialty of the treating physician. The prescription drugs file contains – among others – data on each prescription (including the Drug Identification Number [DIN], the dosage

form and the dose), the date of dispensing at the pharmacy, the duration of the treatment, quantity prescribed and the specialty of the prescribing physician. Importantly, the prescription drugs file contains data on prescriptions dispensed in community pharmacies only, and does not include data on medications dispensed in hospitals.

4.2.2 MED-ECHO

The MED-ECHO database is the Quebec universal hospital discharge summary database that is used in planning, organization and evaluation of services provided in health and social services sectors.[259] The MED-ECHO database covers all residents of Quebec and records data on acute care hospitalizations and same-day surgeries from Quebec's specialized and nonspecialized hospitals and medical centers. Unlike the RAMQ database, MED-ECHO database is used for planning and organization purposes and not for the reimbursement or payment for health professionals.[259] The recorded data from MED-ECHO that were contained within the *Quebec Asthma and Pregnancy Database* included – among others – are the unique patient identifier, the primary discharge, admission and up to 15 secondary discharge diagnoses, the date of entry, the duration of hospital stay and the treatments received during the stay. A complete list of the recorded data in MED-ECHO is available at http://www.ramq.gouv.qc.ca/fr/donnees-statistiques/sur-demande/donnees-msss/Pages/med-echo.aspx#soins. The clinical diagnoses in MED-ECHO are recorded by trained medical archivists using the enhanced version of the International Classification of Diseases (ICD) 10th revision for Canada (ICD-10-CA) since 2006, and using ICD 9th revision (ICD-9) before 2006. For delivery-related hospitalizations, records were retrieved for the gestational age and birth weight of the baby.

4.3 Pregnancies' cohort and linked database

For the two articles on the comparative safety of LABA-ICS combination versus ICS monotherapy and the case ascertainment definitions of major malformations we used pregnancies from the ***Quebec Asthma and Pregnancy Database*** which contains linked data from RAMQ and MED-ECHO. The *Quebec Asthma and Pregnancy Database* includes all pregnancies of all women in Quebec who had ≥ 1 asthma diagnosis (ICD-9: 493 or ICD-10: J45) in the 2-year period preceding one of their deliveries and all pregnancies of a four-

time larger random sample of other women who delivered between January 1, 1990 and March 31, 2010. For each pregnancy included in the cohort, babies were identified (live births and stillbirths) using the mother-child link of the RAMQ. The database includes 583,071 pregnancies from all over Quebec, Canada representing about 35% of all births in the province during these years.[260]

The validity of the diagnosis of asthma recorded in the RAMQ database has been formally validated against the patients' medical charts as gold standard, showing a predictive positive value (PPV) of 75% and a predictive negative value (PNV) of 96% for asthma diagnosis among pulmonologists and 67% and 99% among family physicians.[261] The prescription data recorded in the RAMQ database has been formally evaluated and found to be accurate and valid (83% correct identification of the patients and drugs dispensed from the prescriptions).[262]

4.4 Article on LABA-ICS combination versus ICS monotherapy

4.4.1 Cohort structure and inclusion and exclusion criteria

The flowchart representing the cohort selection is presented in the manuscript in Chapter 5. The cohort was selected using the following inclusion criteria:

1. a pregnancy with a recorded singleton delivery between January 1, 1990 and March 31, 2009, so that at least one year of follow-up data was available for the newborn;
2. at least one asthma diagnosis in the two years preceding delivery (ICD-9: 493 or ICD-10: J45);
3. the use of ICS in the first trimester of pregnancy (1–14 weeks);
4. coverage with the RAMQ's Public Drug Insurance Plan for at least three months before and throughout pregnancy.

We excluded from the analysis pregnancies that met any of the following exclusion criteria:

1. multiple births from a single pregnancy;

2. rare maternal condition affecting fetal development (rheumatic disease, Cushing disease, iodine deficiency, adrenal tumor, and folic acid deficiency) identified using ICD-9 and ICD-10 codes;
3. teratogenic fetal infection;
4. at least one filled prescription for a teratogenic medication in the first trimester;
5. chronic use of an oral corticosteroid (OCS) in the first trimester (i.e., ≥ 30 days' supply);
6. at least one filled prescription for an oral beta$_2$-agonist, leukotriene-receptor antagonist, theophylline, ipratropium, cromoglycate, or nedocromil in the first trimester;
7. for women contributing more than one pregnancy during the study period, we included only the two most recent pregnancies to allow converging regression models.

At the time of the conduct of this project, the work on the teratogens and potential teratogens lists project (included in the current thesis) was not yet concluded. Therefore, we used an updated list of proven teratogenic medications that we previously used for similar projects on congenital malformations risk.[30,216]

Two subcohorts were established to compare the treatment regimens indicated for women with similar levels of asthma severity. In the first subcohort (hereafter referred to as the "moderate asthma subcohort"), we compared women who used LABA plus low-dose ICS with those who used a medium-dose ICS monotherapy. In the second subcohort (hereafter referred to as the "severe asthma subcohort"), we compared women who used LABA plus medium-dose ICS with those who used a high-dose ICS monotherapy. The final total cohort included 1302 pregnancies (in 1249 women), 948 pregnancies in the moderate asthma subcohort and 354 in the severe asthma subcohort. In this study, LABA plus high ICS dose users and low ICS monotherapy users were excluded.

4.4.2 Exposures assessment

For both ICS and LABA exposure assessments, we used data from the RAMQ database on dispensed prescriptions at community pharmacies. For the ICS exposure

(fluticasone, beclomethasone, triamcinolone, flunisolide, budesonide, or ciclesonide), since the average doses used by patients can differ from one another, we estimated the average daily dose taken during the first trimester, because this is considered the period of highest fetal risk where the majority of organs and systems develop. The estimate was made using an algorithm that was developed by our research team and used for previous studies.[34] The algorithm is based upon the name of the medication, the equivalence between the different ICS products recognized by the Canadian Asthma Consensus Guidelines (in fluticasone equivalents), [82] the dose prescribed, the date and duration of the filled prescription, and the rate of renewal of the prescription. The daily dose of ICS was categorized as follow: low dose (> 0–250 μg), medium dose (> 250–500 μg), and high dose (> 500 μg).

LABA (salmeterol and formoterol) are not used as commonly as ICS and their prescribed doses do not vary as it is for ICS. Therefore, LABA use was defined as filling at least one prescription during the first trimester or three months before pregnancy, with the likelihood of its use during the first trimester based on the date and duration of the filled prescription, where the duration of the prescription is required to overlap with the beginning of the pregnancy to be considered as exposed during the first trimester. Due to the established safety evidence on ICS monotherapy from previous reports, compared to the smaller body of evidence on LABA, we chose to use ICS monotherapy in higher doses as the reference group.

4.4.3 Outcomes definition

The primary outcome was any major congenital malformation. Full details on the outcome definition is presented in the manuscript in Chapter 5. Briefly, cases of major congenital malformations were identified using the ICD-9/ICD-10 hospital-based diagnostic codes recorded in the RAMQ or MED-ECHO databases at birth or during the first year of life of the infant. The specific major malformation classes and their related diagnostic codes are presented in Table E1 in the manuscript in Chapter 5. We used the Two-step Congenital Malformation Classification (TCMC) method which is presented in full details in the article on case ascertainment definitions of major malformations (see below). A congenital malformation was defined as major if it was life threatening or could cause major cosmetic defects. When a malformation could be classified as major or minor

by the geneticist, we considered it as major only if there was at least one hospitalization with a primary diagnosis or admission diagnosis related to this malformation that was recorded in the MED-ECHO database during the first year of life of the newborn.

4.4.4 Statistical analysis

Using descriptive statistics, we reported and compared the characteristics of the pregnancies for the LABA-ICS combination group and the ICS monotherapy group within each subcohort. We calculated the crude prevalence of any major or a specific major malformation within each subcohort. The cohort of ICS users comprised 6 mutually exclusive groups: 1) LABA-ICS low dose, 2) LABA-ICS medium dose, 3) LABA-ICS high dose, 4) ICS low dose monotherapy, 5) ICS medium dose monotherapy, and 6) ICS high dose monotherapy. The pregnancies from ICS low dose monotherapy users and LABA-ICS high dose users were excluded. Using the ICS higher dose monotherapy as the reference group (due to the established evidence on their safety from previous reports), the risk of major congenital malformations was compared between the LABA-ICS combination therapy and ICS monotherapy separately within the two subcohorts. We used the pregnancy as the unit of analysis. Generalized estimating equation (GEE) models with a logistic link and an exchangeable correlation matrix were used to estimate the crude and adjusted odds ratios for major congenital malformations, while adjusting for all of the potential confounders (full models) listed in Table 4.6. The GEE models were used as they take into account the correlation between the consecutive pregnancies of individual women.[263] After pre-hoc testing using the unstructured, independent and exchangeable correlation matrices, we chose to use the exchangeable matrix since the theoretical assumptions behind it are adequate for our situation, the results were similar for the three matrices and better stability was achieved using it (additional details on the correlation matrices is presented below; 4.6.2).

In an attempt to increase the power of the analysis, compared to our primary stratified analysis, a sensitivity analysis combining the two subcohorts together while adjusting for a variable indicating from which subcohort the pregnancy came was performed. The indicator variable identifies if the pregnancy came from the severe asthma subcohort (yes) or the moderate asthma subcohort (no). The combined adjusted results

from this analysis represent an overall comparison of the LABA-ICS combination therapy and the ICS monotherapy in higher-doses. All statistical analyses were performed with the SAS software, version 9.3 (SAS Institute Inc., Cary, NC).

For the power calculations, we performed *post hoc* power calculations to identify the ORs that each analysis could detect with a power of 80%. These calculations were based on a test for the difference between two independent proportions with a type I error of 0.05, the number of pregnancies exposed to each of the contrasted treatment regimens in the subcohorts, and the percentage of pregnancies with a congenital malformation observed in the reference group (higher-dose ICS monotherapy). Power calculations were performed using the PASS interface of the NCSS software (2007). No additional statistical analysis, beside the ones published in the manuscript, were performed.

4.5 Article on case ascertainment definitions of major malformations

4.5.1 Cohort structure and inclusion and exclusion criteria

The flowchart representing the cohort selection is presented in the manuscript in Chapter 5. The cohort was selected using the following inclusion criteria:

1. a pregnancy with a recorded delivery between January 1, 1990 and March 31, 2009, so that at least one year of follow-up data was available for every newborn;
2. maternal age at the beginning of pregnancy of 15–45 years;
3. gestational duration of 20–45 weeks;
4. fulfillment of the definitions for the presence or absence of active asthma during pregnancy (definitions presented below in subsection 4.5.2)

We excluded from the analysis pregnancies that met any of the following exclusion criteria:

1. Quadruplet births from a single pregnancy;
2. pregnancies missing the mother–infant link.

The final cohort used for the analysis included 467,946 pregnancies, 57,766 (12.3%) were in women with active asthma and 410,180 (87.7%) were in non-asthmatic women.

4.5.2 Exposures assessment

The secondary objective of that study was to evaluate the impact of different case ascertainment definitions on the association between maternal asthma and major congenital malformations. Therefore, we selected an operational definition for active asthma during pregnancy to apply in the statistical analysis. This operational definition of asthma was previously validated and showed a sensitivity of 83.8% and a specificity of 76.5%.[264] Asthma was defined as ≥ 1 asthma diagnosis (ICD-9 code 493, except 493.2, or ICD-10 code J45) recorded during a hospitalization, or ≥ 2 medical claims associated with an asthma diagnosis within 2 consecutive years between 1988 and the delivery. Asthma was considered active during pregnancy if ≥ 1 medical service for asthma was recorded in the RAMQ or MED-ECHO databases up to 2 years before delivery. The pregnancy was considered as non-asthmatic (i.e. the reference group) if the woman had no diagnosis of asthma recorded in either database between 1988 and the delivery. The use of active asthma definition in this study differs from the study on LABA-ICS combination vs ICS monotherapy because the later study had another important inclusion criterion not applicable to the current study, which is ICS use in the first trimester.

4.5.3 Outcomes definition

The article investigates the impact of different case ascertainment definitions of major congenital malformations on the prevalence estimates observed and on maternal exposure-outcome association estimates (i.e. maternal asthma as exposure). The manuscript presented in Chapter 5 contains the full details of the different case ascertainment definitions that were examined. Briefly, we compared two methods for the classification of congenital malformations. The first, the Two-step Congenital Malformation Classification (TCMC) method, which was developed specifically for research and used in previous perinatal pharmacoepidemiologic studies.[34,216]The second method was the national Canadian Congenital Anomalies Surveillance System (CCASS) method. Table e1 in the

manuscript provides a complete description and comparison of the two classification methods. In the study, we aimed to compare case ascertainment definitions that differ by the source of data (i.e. diagnoses recorded in a hospital database [MED-ECHO] or in a medical billing claims database [RAMQ]) and the classification method (i.e. the TCMC or CCASS methods). We compared six different case ascertainment definitions (detailed in Table 1 in the manuscript in Chapter 5).

4.5.4 Statistical analysis

The characteristics of the pregnancies were compared between the pregnancies of women with active asthma and non-asthmatic women using descriptive statistics. The prevalence of congenital malformations was defined as follows: prevalence = number of pregnancies with at least one malformation among live births and stillbirths/total number of pregnancies with live births and stillbirths.

Using the six different case ascertainment definitions, we calculated the prevalence of major malformations and system-specific categories of major malformations. Then, using pregnancy as the unit of analysis, we compared the prevalence of major congenital malformations between pregnancies of women with active asthma and non-asthmatic women (maternal asthma-major malformations association). We used GEE models with a logistic link and the exchangeable correlation matrix to estimate crude and adjusted odds ratios for major malformations with 95% confidence intervals. The adjusted models included the list of potential confounders (full models) listed in Table 4.6.

In a sensitivity analysis not published in the article by Eltonsy et al. (published in MCHJ; 2016), we sought to examine the effect of the choice of the correlation matrix in the GEE models on the point estimates and the confidence intervals estimated. In the application of the GEE models, the user specifies a working correlation structure for describing how the responses within clusters are related to each other.[265,266] Correlation structures that are commonly considered include independent, exchangeable, autoregressive, stationary, unstructured, and fixed. In congenital malformations research, independent, exchangeable, and unstructured matrices can be reasonable choices, due to the plausible assumptions they carry. The assumption behind the independent correlation structure is that responses are uncorrelated within a cluster.[265] The assumption behind the

use of the exchangeable correlation structure is that any two responses within a cluster have the same correlation.[265] In an unstructured correlation structure, there are less constraints on the correlation parameters. An unstructured correlation structure has a separate correlation parameter for each pair of observations within a cluster, even if the time intervals between the responses are the same. For a cluster that has n responses, there are n(n-1)/2 correlation parameters. The presence of such large number of correlation parameters to be estimated in the unstructured correlation structure makes it one of the most complex correlation structures.[265] Although GEE models can provide relatively valid standard errors, even when the correlation structure is incorrectly specified, it is interesting to examine the results of the GEE models when using different correlation matrices that differ by complexity. In this sensitivity analysis, we used exchangeable, independent, and unstructured correlation matrices in the GEE models, and compared the results obtained using the three matrices. All statistical analyses were performed with SAS software, version 9.3 (SAS Institute Inc., Cary, NC). The results are presented in Chapter 5 results, section 5.3.

4.6 Potential confounders

4.6.1 Risk factors for major congenital malformations

The different risk factors for congenital malformations retrieved from our literature review are presented in Chapter 2, section 2.7. The main reason behind excluding several of the risk factors in our multivariate models is the absence of these variables from the RAMQ and MED-ECHO databases (e.g. maternal smoking status and folic acid use). Some other risk factors had very low prevalence among the pregnancies included in our cohorts, which hindered their inclusion in our models. For example, rare maternal conditions (as rheumatic diseases and phenylketonuria) which had low prevalence in our cohort. The details of the excluded conditions are presented in section 4.3 and chapter 5.

4.6.2 Potential confounders included in the statistical analysis

Table 4.6 Potential confounding variables included in the statistical analysis

Confounding variable	Article on LABA-ICS combination versus ICS monotherapy	Article on case ascertainment definitions of major malformations	Database used in identification
Maternal sociodemographic characteristics			
Maternal age at the beginning of pregnancy (18–34 and <18 or ≥35 years)	X	X	RAMQ
Area of residence at delivery (rural/urban)	X	X	RAMQ
Receipt of social assistance at the beginning of pregnancy (yes/no)	X	X	RAMQ
Maternal comorbidities and pregnancy related characteristics			
Chronic hypertension up to 1 year before pregnancy[§]	X	X	RAMQ and MED-ECHO
Diabetes mellitus up to 1 year before pregnancy[§]	X	X	RAMQ and MED-ECHO
Maternal epilepsy up to 1 year before pregnancy[§]		X	RAMQ and MED-ECHO
Multiple pregnancy		X	RAMQ and MED-ECHO
Asthma related variables			
exacerbation of asthma three months before pregnancy (yes/no) *	X		RAMQ and MED-ECHO
SABA doses per week in the three months preceding pregnancy[¥]	X		RAMQ

[§] Identified using the ICD-9/ICD-10 diagnoses codes recorded in the MED-ECHO or RAMQ databases up to 1 year before pregnancy

* Defined as a filled prescription for OCS, an emergency department visit, or a hospitalization for asthma

[¥] Classified into 0–3 or > 3 doses/week; one dose is equal to 200 μg of salbutamol

A note on our confounders selection; we based our choices on the established definitions of confounding. Typically, a spurious association appears due to the sharing of common causes (i.e. a confounder, given that it is not in the causal pathway between the exposure and the outcome).[267] In the article on the LABA-ICS combination versus ICS monotherapy in higher doses, several of the risk factors identified in Chapter 2, section 2.7 do not meet these criteria. For example, while alcohol consumption is considered a strong risk factor for congenital malformations, there is no data suggesting that alcohol use has a differing prevalence between LABA-ICS combination users and ICS monotherapy users in high doses.

In the second article on the different case ascertainment definitions of major malformations, the true association between asthma and major malformations is not the objective of the study, but rather how the estimates are affected by the differing case ascertainment definitions. Therefore, better statistical stability was one of our primary goals.

4.7 Systematic Procedure for the Classification of Proven and Potential Teratogens

This section covers the methodology details that were not reported in the manuscript presented in Chapter 5.

4.7.1 Steps and settings

We developed a systematic two-step procedure for teratogen identification and classification for research purposes. By applying the procedure, two medication lists can be obtained, one including “teratogenic medications” and the other including “potentially teratogenic medications”. Full details on the two-step procedure is presented in the published article in Chapter 5.

Briefly, Step 1 included the identification and classification of medications reported in the reference book Drugs in Pregnancy and Lactation: a Reference Guide to Fetal and Neonatal Risk (9th ed.) by Briggs et al. 2011 (Briggs and others, 2011) into two provisional lists: 1) teratogenic medications, and 2) potentially teratogenic medications. Followed by a review by a teratology expert (B.M.) leading to either the approval of classification or further verification, i.e., entry on a "verification list". Other references were searched, including reviews of teratogenic drugs and drug-related birth defects, textbooks of teratogenicity, and Briggs et al. updates (till October 2013), to identify other potential teratogens to be added to the verification list.

In Step 2, we searched the TERIS database for the medications in the verification list. The details of the procedures applied in Step 2 are presented in the manuscript in Chapter 5. Briefly, we searched the TERIS database for each medication in the verification list, and if the medication was present, we classified it according to the newly developed "TERIS scheme" as presented in the manuscript (Chapter 5). If the medication was absent from the TERIS database, we classified it based on our "expert consensus". The expert consensus was the opinion of two experts in teratogenicity and reproductive risk (B.M. and E.F.). The experts used all available published reports and resources to develop their ratings. For the inclusion into List 1, the experts used the criteria for proof of human teratogenicity proposed by Shepard and presented below in Table 4.8.[219] The experts' opinions were collected by a third author and a consensus meeting was conducted to resolve any conflicting decisions.

Table 4.7: Shepard's amalgamation of criteria for proof of human teratogenicity (Source: Shepard, 1994)[219]

(1) Proven exposure to agent at critical time(s) in prenatal development.
(2) Consistent findings by two or more epidemiologic studies of high quality:
(a) Control of confounding factors
(b) Sufficient numbers
(c) Exclusion of positive or negative bias factors
(d) Prospective studies, if possible
(e) Relative risk of six or more
(3) Careful delineation of the clinical cases. A specific defect or syndrome, if present, is very helpful
(4) Rare environmental exposure associated with rare defect
(5) Teratogenicity in experimental animals
(6) The association should make biological sense
(7) Proof in an experimental system that the agent acts in an unaltered state Evidence of placental transfer

Note: items (1), (2), and (3) or (1), (3), and (4) are essential criteria. Items (5), (6), and (7) are helpful but not essential.

For a medication to be included into List 2 (potentially teratogenic medications), the experts used three stepwise conditions that the potential teratogen has to fully satisfy:

1st Step. The experts verified that the medication did not meet Shepard's criteria (if it meets the criteria: send back to List 1, if no: proceed to Step 2).

2nd Step. The experts examined if enough evidence exists that suggest the absence of a teratogenic risk in humans (if yes: to not include in neither list, if no: proceed to Step 3).

3rd Step. The experts examined if there is 1 human study or sufficient animal data that shows evidence of teratogenic risk (if yes: to include the medication in List 2, if no: to not include in neither list).

4.7.2 Statistical analysis

We tallied the number of medications included in each step with our classification procedure. We calculated the number and percentage of observed agreements between the two experts in teratogenicity. We also calculated the kappa value, with its 95% confidence interval (CI), and the weighted kappa for the agreement between the two experts. The calculation of weighted kappa assumes the categories are ordered and accounts for how far apart the two raters are.[268] The following table was used to interpret the *K* value:

Value of *K*	Strength of agreement
≤ 0.20	Poor
0.21 - 0.40	Fair
0.41 - 0.60	Moderate
0.61 - 0.80	Good
0.81 - 1.00	Very good

Source: Altman DG (1999) Practical statistics for medical research. London: Chapman and Hall.

Measures of agreement were calculated with GraphPad Prism 2015 (GraphPad Software Inc. 2015, La Jolla, CA, USA).

4.8 Ethical approval

4.8.1 Systematic review on beta$_2$-agonists and perinatal outcomes

Primary data was not collected. The review did not involve any human or animal subjects (including human material or human data). Because the study was conducted using online resources and research databases, no ethical committee approval was required.

4.8.2 Article on LABA-ICS combination versus ICS monotherapy

This research project was approved by the Ethics Committee of the *Hôpital du Sacré-Cœur de Montréal*. Authorization was obtained from the *Commission d'Accès à l'Information du Québec* to access and link the RAMQ and MED-ECHO databases.

4.8.3 Article on case ascertainment definitions of major malformations

This research project was approved by the Ethics Committee of the *Hôpital du Sacré-Cœur de Montréal*. Authorization was obtained from the *Commission d'Accès à l'Information du Québec* to access and link the RAMQ and MED-ECHO databases.

4.8.4 Systematic Procedure for the Classification of Proven and Potential Teratogens

Because the study was conducted using online resources and medical references, and did not involve any human or animal subjects (including human material or human data), no institutional review board approval was required.

CHAPTER 5: RESULTS - MANSCRIPTS

Results and Manuscripts

5.1 Systematic review on beta$_2$-agonists and perinatal outcomes

Beta$_2$-agonists use during pregnancy and perinatal outcomes: A systematic review

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BETA$_2$-AGONISTS USE DURING PREGNANCY AND PERINATAL OUTCOMES: A SYSTEMATIC REVIEW

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Abstract

Background: Short and long-acting beta$_2$-agonists (SABA and LABA) have a crucial role in asthma management during pregnancy, as stated in the current guidelines. **Objective**: To systematically review the evidence on beta$_2$-agonists use during pregnancy and adverse perinatal outcomes. **Data sources and study selection**: Six databases were searched before January 1, 2013 for beta$_2$-agonists use during pregnancy and congenital malformations, small for gestational age, mean and low birth weight, gestational age and preterm delivery. Original English language articles were included with no cut-off date. Quality assessment and post-hoc power calculations were performed. **Results**: Twenty-one original studies were identified. Four studies reported a significant increased risk of congenital malformations with SABA, while one study reported a significant decreased risk with high doses of SABA. One study reported a significant increased risk of congenital malformations with LABA and four studies reported a significant increased risk of congenital malformations with beta$_2$-agonists (SABA and/or LABA). One study reported a decrease in birth weight centiles among LABA users. **Limitations**: All studies reporting significant results, except two, used non-asthmatic women as reference group, making it difficult to differentiate between the effect of the disease from the one of the beta$_2$-agonists. Non-significant results should be interpreted with caution due to the low statistical power of several studies. **Conclusion**: Methodological limitations and lack of power of several studies prevent us to conclude on the perinatal safety of beta$_2$-agonists. Until further evidence is available, physicians should continue prescribing them as recommended in the guidelines whenever needed to attain asthma control.

Key words: Asthma, Pregnancy, Bronchodilators, Beta-2-agonists, Birth weight, Congenital defects, Gestational age, Preterm birth.

1. Introduction

Asthma is considered to be one of the most common chronic diseases among pregnant women, affecting approximately 4 to 8% of the pregnancies in the United States and even higher among other populations.[1-3] Pregnant women with severe or uncontrolled asthma are at higher risk for pregnancy complications and adverse fetal outcomes than women with well-controlled asthma.[2,4-8] Due to the reported potential risk of uncontrolled asthma during pregnancy on the health of the mother and fetus, the National Asthma Education and Prevention Program (NAEPP) states that "(…) it is safer for pregnant women with asthma to be treated with asthma medications than it is for them to have asthma symptoms and exacerbations."[2]

While inhaled corticosteroids (ICS) are considered the cornerstone therapy in the management of persistent asthma during pregnancy,[9,10] $beta_2$-agonists have a crucial role in asthma management.[2] During pregnancy, short-acting $beta_2$-agonists (SABA) are used as reliever medications for all asthma types (mild, moderate, or severe), while long-acting $beta_2$-agonists (LABA) are used in cases of moderate to severe persistent asthma, in combination with low or medium doses of ICS.[2,11] It has been reported that 40 to 70% of asthmatic women use SABA and 8 to 13% use LABA during pregnancy.[12,13] Despite being widely used during pregnancy, all of the SABA and LABA are classified as "C" under the US Food and Drug Administration (FDA) categorization[4], which states that risk cannot be ruled out and that there is a chance of fetal harm if the drug is administered during pregnancy, but the potential benefits may outweigh the potential risk. Moreover, the Teratogen Information System (TERIS) reports that SABA and LABA have an "Undetermined" teratogenic risk due to the limited quality and quantity of data on the safety of these medications [4].

Several studies examined the effect of SABA and LABA use on perinatal outcomes during pregnancy.[2,14-24] Published reviews on this topic did not capture the whole evidence from all published studies on all clinically important perinatal outcomes. [4,14-16,25,26] Given the need to better estimate their fetal risks, we aimed to summarize the existing human data - from experimental trials and observational studies - examining the impact of the use of inhaled SABA and LABA for the treatment of asthma during pregnancy on several perinatal outcomes, which are major and any congenital malformations, small for gestational age (SGA; weight $\leq 10^{th}$ percentile for the gestational age), birth weight, low

birth weight (LBW; weight <2500 g), gestational age and preterm delivery. We also assessed the quality of each study using a validated quality assessment scale and performed post-hoc power calculations to evaluate the capacity of the studies to detect clinically relevant effects.

2. Methods

2.1 Data Sources and Search Strategy

A search strategy was formed, registered and published (PROSPERO 2011:CRD42011001554, http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42011001554). PubMed, Ovid MEDLINE, EMBASE, Cochrane Library, Web of Science, and CINAHL were searched for original articles. The first search was performed using the keywords "asthma*" and "pregnan*". A second search was done using the keywords "congenital", "malformations", "congenital anomalies", "birth weight", "low birth weight", "small for gestational age", "gestational age", "preterm delivery", "preterm birth", "embryonic development", "fetal development" and "foetal development" , combined with "asthma*" and "pregnan*". A third search was conducted using keywords "beta-agonist", "short-acting beta-agonist", "long-acting beta-agonist" and the individual medication names [salbutamol, albuterol, terbutaline, metaproterenol, fenoterol, salmeterol, and formoterol], together with "asthma*" and "pregnan*". Furthermore, we applied a cross-search using the keywords in the three searches. A Medical Subject Heading (MeSH) search was also conducted in MEDLINE, using the terms "asthma" and "pregnancy". Human studies published in English language were only considered in our final selection and no particular cut-off for the date of publication was used. Only original articles were included and abstracts without supporting articles were excluded. No exclusion criteria were imposed on either the choice of the reference groups or the treatments compared to $beta_2$-agonists. All types of studies (RCTs, case-control and cohort studies) were searched except case-reports, case-series and Prescription-Event Monitoring studies (PEM). All inhaled $beta_2$-agonists were included either taken separately or in combination with ICS. The latest search was performed on January 1, 2013. Related articles and data cited in the reference book "Drugs in Pregnancy and Lactation: A Reference Guide to Fetal and Neonatal Risk"[17] were also

included. Bibliographies of all retained articles and reviews on the topic were searched for additional relevant articles.

2.2 Data Extraction and Study Selection

The search strategy, including the inclusion and exclusion criteria, is summarized in Figure 1. We chose seven outcomes that we believe best represent the fetal development (major and any malformations, SGA, mean and low birth weight) and the newborn prematurity (gestational age and preterm delivery) among asthmatic women treated with beta$_2$-agonists. The primary search was conducted by one author (SE), while a second confirmatory independent search was performed by a second author (FZK). All studies identified in the search were independently reviewed by two co-authors and the study selection was made independently by two co-authors (SE and FZK). Data extraction, quality assessment and post-hoc power calculations were first performed by one author (SE). An independent data extraction and power calculation were performed by a second author (FZK). Discrepancies were resolved by consensus.

Data retrieved from each study included the study reference, the design, the source of data, the timing of exposure, the type of beta$_2$-agonists, the definition of the reference group, the sample size of the exposed and unexposed groups, the reported proportions or means and standard deviations for the outcomes in the exposed and unexposed groups, the effect size (crude or adjusted relative risk [RR], odds ratio [OR], or mean difference [MD]), and the p-value or 95% confidence interval (CI) associated with the effect size. In studies that did not report the effect size, a crude RR, OR, or MD was calculated when sufficient information was provided.

2.3 Quality Assessment and Power Calculation

The Newcastle-Ottawa Scale for observational studies (NOS-scale) was used for assessing the methodological quality of studies that passed the defined inclusion criteria.[27] We used the NOS-scale based on recommendations by the Cochrane Non-Randomized Studies Methods Working Group since all of the studies included were expected to be non-randomized.[28] The NOS-scale has two forms, one for cohort studies and one for case-control studies, and studies are being judged on three domains: 1) selection of study groups

(score:0-4), 2) comparability of the groups (score:0-2), and 3) exposure/outcome ascertainment (score:0-3). An external reviewer was called for the quality assessment of the study published by our group [29] in order to avoid a conflict of interest.

We performed a post-hoc power calculation for each study reporting non-statistically significant results to detect a RR of 1.5, a mean difference in the birth weight of 500 g, or a mean difference in gestational age of one week to establish a comparison between studies. The power calculations were based on t-tests for MD and on the test for the difference between two independent proportions for RR and OR. A type I error of 0.05 was used for power calculations; all calculations were performed using PASS 2008 interface of NCSS software.[30]

3. Results

3.1 Study Selection

Study selection results are summarized in Figure 1. Using our selection criteria, 19 original studies were found.[18-24,29,31-41] After reviewing all cited references in the retrieved studies, we added the data from the Collaborative Perinatal Project (CPP) and the Michigan Medicaid study (their data were retrieved from 2 books[17,42], see Figure 1.), having a total of 21 studies included in our review.[17-24,29,31-42] We did not exclude any studies even if they provided insufficient information for the power calculation. Thirteen were cohort studies[17-21,23,24,29,33,34,36,39,42], seven were case-control studies[31,32,35,37,38,40,41], and one was a cohort study that contained partial data from a randomized controlled trial.[22] Nine studies reported statistically significant results.[24,29,31,32,36,37,40-42] Post-hoc power calculations were performed for certain outcomes in eighteen studies [18-24,29,31-35,37-41], while the lack of information prevented us from performing power calculations for certain outcomes in four studies.[17,19,36,42] Results from the quality assessment of the studies using the NOS-scale are summarized in Table 1.

3.2 Major congenital malformations

Major congenital malformations are defined as structural and developmental anomalies that affect viability and/or quality of life and require intervention.[43]

Studies that investigated the association between beta$_2$-agonists and major congenital malformations are presented in Table 2A: six studies examined SABA and/or LABA use[20,22,31,32,40,41], nine studies examined the SABA separately[21,23,29,31,32,35,36,40,41], and six studies examined LABA separately.[29,32,35,36,40,41]

Among the six studies that investigated SABA and/or LABA use, two studies used a reference group of asthmatic pregnant women unexposed to beta$_2$-agonists during pregnancy; none of these two studies reported a significant increased risk of major malformations.[20,22] A reference group of non-asthmatic or a combination of asthmatic and non-asthmatic pregnant women has been used in the other studies[20,31,32,40,41], with four studies reporting a significant increased risk of major malformations.[31,32,40,41] Indeed, Lin et al. in three studies reported increased risk of congenital heart defects[31] (aOR=2.20; 95% CI: 1.05, 4.61), gastroschisis[32] (aOR=2.06; 95% CI: 1.19, 3.59), and other selected birth defects[41] (aOR=2.39; 95% CI: 1.23, 4.66) with beta$_2$-agonists use during the first trimester. In a recent case-control study by Munsie et al. an association between bronchodilator use (mainly SABA and LABA) and an increased risk of cleft lip only was found (aOR=1.77; 95% CI: 1.08, 2.88).[40]

Among the nine studies that evaluated SABA separately, three studies used asthmatic women unexposed to SABA during pregnancy as the reference group[23,29,35] while the other six studies used non-asthmatic women or the general population as the reference group.[21,31,32,36,40,41] In a study by Kallen et al. using the Swedish Medical Birth Registry, the authors reported an increased risk of any cardiac defect with salbutamol use in the first trimester (aOR=1.38; 95% CI 1.12, 1.70) when compared to the general population.[36] In the study by Munsie et al., the authors found a significant increased risk of cleft lip (aOR=1.79; 95% CI: 1.07, 2.99) and cleft palate (aOR=1.65; 95% CI: 1.06, 2.58) with the maternal use of salbutamol during the periconceptional period as compared to the general population of asthmatics and non-asthmatics [40]. In a recent study by our group, we reported a decreased risk of major malformations with the maternal use of high doses of SABA per week (>10 doses) as compared to no use, with an aOR of 0.68 (95% CI: 0.48, 0.95).[29] None of the other five studies reported a significant association.

Regarding the six studies that examined LABA use separately, two used a reference group of asthmatic women unexposed to LABA.[29,35] In the study conducted by our research group, we found a significant increased risk of major cardiac (aOR=2.38; 95% CI: 1.11,

5.10) and major "other and unspecified malformations" (aOR=3.97; 95% CI: 1.29, 12.20) among LABA users compared to asthmatic non users, but the association rendered non-significant when all major malformations were combined together.[29] None of the other five studies reported a significant association between LABA use and major malformations.[32,35,36,40,41]

The ten studies that reported non-significant results for major congenital malformations and for which we had enough information to calculate the statistical power had a power ranging from 6% to 100% to detect an effect size of 1.5, with only 2 studies having a power > 80%.[35,41]

3.3 Any congenital malformations

Congenital malformations can be defined as any structural or functional anomalies, including metabolic disorders.[43] Any congenital malformations include all types of congenital malformations (major or minor) that could occur during fetal development.

Studies that investigated the association between beta$_2$-agonists use during pregnancy and any congenital malformations are presented in Table 2B: four studies examined SABA and/or LABA use[19,33,34,39], six studies examined SABA separately[17,29,36-38,42], and four studies examined LABA separately.[29,36-38] None of the four studies that investigated SABA and/or LABA use reported significant results; only two studies used a reference group of asthmatic pregnant women unexposed to beta$_2$-agonists during pregnancy.[19,33]

Among the six studies that evaluated the effect of SABA separately, two studies used asthmatic women unexposed to SABA during pregnancy as the reference group,[29,37] and a significant association was found in one study.[37] In this matched case-control study, Tamasi et al. reported a significant increased risk of any malformations with maternal use of fenoterol during the first trimester of pregnancy (crude OR=1.6; 95% CI: 1.3, 2.0).[37] Reference groups formed by the general population, non-asthmatic pregnant women, or unspecified reference groups have been used in four studies[17,36,38,42] and significant associations were reported in two of these studies.[36,42] A significant increased risk of any malformations with the use of epinephrine (SABA) during the first trimester was reported by the CPP group (RR=1.7, P<0.05)[42]. Kallen et al. in a retrospective cohort study using

the Swedish Medical Birth Registry reported a slight increased risk of any malformations with terbutaline use in the first trimester of pregnancy (aOR=1.11; 95% CI:1.04, 1.19).[36]

From the four studies that examined the use of LABA separately, two used a reference group formed of asthmatic women unexposed to LABA during pregnancy[29,37], one used a reference group formed of asthmatic and non-asthmatic women[38], and the reference group was the general population in one study,[36] Only one of these studies found a significant result: women exposed to LABA were found to have an increased risk of genital malformations (aOR=6.84; 95% CI: 2.58, 18.10) and "other and unspecified malformations" (aOR=3.43; 95% CI: 1.39, 8.45) when compared to asthmatic women unexposed to LABA during pregnancy.[29]

The six studies that reported non-significant results for any congenital malformations and provided enough information to calculate the statistical power had a power ranging from 9% to 100% to detect an effect size of 1.5 with three studies having a power > 80%.[29,37,38]

3.4 Small for gestational age

Studies that investigated the association between beta$_2$-agonists use during pregnancy and SGA are presented in Table 3: three studies examined SABA and/or LABA use[19,20,22], three studies examined SABA separately,[18,21,23] and two studies examined LABA separately.[18,24] The three studies that examined the use of SABA and/or LABA used a reference group of asthmatic pregnant women unexposed to beta$_2$-agonists during pregnancy [19,20,22], and none of these studies reported a significant increased risk of SGA with beta$_2$-agonists use. Among the three studies that examined SABA separately, only one used a reference group formed of asthmatic women unexposed to SABA[23]; and the three studies reported non-significant associations between SABA exposure and SGA. Among the two studies that examined LABA separately, one study used a reference group formed of asthmatic women unexposed to LABA during pregnancy [24] and both studies reported non-significant results.

The six studies that reported non-significant results for SGA and for which we had enough information to calculate the statistical power had a power ranging from 8% to 60% to detect an effect size of 1.5.

3.5 Birth weight and low birth weight

3.5.1 Birth weight

Studies that investigated the association between beta$_2$-agonists and birth weight are presented in Table 4A: four examined SABA and/or LABA use[19,20,34,39], one examined SABA separately[23], and one examined LABA separately.[24] Among the four studies that examined SABA and/or LABA use, two used a reference group formed of asthmatic women unexposed to beta$_2$-agonists during pregnancy [19,20]; none of these four studies reported a significant association between beta$_2$-agonists use during pregnancy and mean birth weight. The only study that investigated the association between the use of SABA separately and birth weight did not find a significant difference in the mean birth weight between the compared groups.[23] The only study that examined the association between the use of LABA separately and birth weight did not find a significant association with the mean birth weight, but found a significant decrease in the birth weight centiles among women exposed to salmeterol when compared to women exposed to budesonide during pregnancy (cMD = -39.2; P-value: 0.011).[24]

The four studies that reported non-significant results for birth weight and for which we had enough information to calculate the statistical power had a power of 100% to detect a mean difference of 500g.

3.5.2 Low birth weight

Studies that investigated LBW are presented in Table 4B: three studies examined SABA and/or LABA use[22,33,34], and two studies examined SABA separately.[21,23] Among the three studies that examined SABA and/or LABA use, two of them used a reference group of asthmatic women unexposed to beta$_2$-agonists[22,33], and none of these three studies reported a significant association between beta$_2$-agonists use during pregnancy and LBW. Among the two studies that focused on SABA, one used two references groups, one formed of asthmatic women unexposed to SABA and one formed of non-asthmatic women[23], and the other one used a reference group formed of non-asthmatics.[21] Both studies found no significant association between SABA use and LBW.

The four studies that reported non-significant results for LBW and for which we had enough information to calculate the statistical power had a power ranging from 3% to 67% to detect an effect size of 1.5.

3.6 Gestational age and preterm delivery

3.6.1 Gestational age

Studies that investigated the impact of beta$_2$-agonists on gestational age at birth (in weeks) are presented in Table 5A: three studies investigated the impact of SABA and/or LABA use[20,34,39] and one study examined LABA separately.[24]Among the three studies that examined SABA and/or LABA use, only one used a reference group formed of asthmatic women (ICS users)[20], and none of the three studies found a significant association between beta$_2$-agonists and gestational age. The only study that examined LABA separately did not find any significant association between LABA use and gestational age.[24]

The four studies that reported non-significant results for gestational age had a power to detect a mean difference of one week ranging from 90% to 100%.

3.6.2 Preterm delivery

Studies that investigated the impact of the use of beta$_2$-agonists on preterm delivery (<37 weeks) are presented in Table 5B: three examined SABA and/or LABA use[22,33,34], two examined SABA separately[18,23], and one examined LABA separately.[18] Among the 3 studies that examined SABA and/or LABA use, 2 studies used a reference group formed of asthmatic women unexposed to beta$_2$-agonists during pregnancy [22,33]; none of these three studies reported a significant association between beta$_2$-agonists and preterm delivery. One of the two studies that examined SABA separately used a reference group of asthmatic women unexposed to SABA during pregnancy[23] and both studies reported non-significant associations with preterm delivery. Moreover, the study that examined LABA separately used a reference group of asthmatic and non-asthmatic women unexposed to LABA; and did not find a significant association between LABA and preterm delivery.

The five studies that reported non-significant results for preterm delivery and for which we had enough information to calculate the statistical power had a power ranging from 12% to 76% to detect an effect size of 1.5.

4. Discussion

We described 21 studies that investigated the impact of beta$_2$-agonists use during pregnancy on perinatal outcomes. Eight studies reported a significant increased risk of congenital malformations for women exposed to SABA and/or LABA[31,32,40,41], SABA separately[36,37,40,42], or LABA separatlety[29] during pregnancy. On the other hand, one study found a significant decreased risk of major malformations with high doses of SABA.[29] No significant associations were reported between SABA and LABA and all other perinatal outcomes, with the exception of one study that reported a significant decrease in birth weight centiles among salmeterol (LABA) users.[24] We observed no impact of beta$_2$-agonists on the risk of preterm birth despite that they can inhibit uterine contractions and be used in IV formulation to control premature labour.[44] This negative result might be explained by the fact that the drug profile of inhaled beta$_2$-agonits shows very low detectable plasma levels with the administration of recommended doses, and consequently minor – if not negligible – tocolytic effect.[45-48]

It is worth noting that six of the eight studies reporting significant increased risk of congenital malformations used a reference group of non-asthmatic women or a combination of asthmatic and non-asthmatic women, studies in which it becomes impossible to separate the effect of the medication from the disease. [31, 32, 36, 40-42] The other two studies used a reference group of unexposed asthmatic women during pregnancy. [29,37] The first reported an increased risk of any congenital malformations with fenoterol (SABA) during pregnancy (crude OR=1.6; 95% CI: 1.3, 2.0), but no association with other SABA. Self-reported questionnaires were used to classify cases (n=511) and controls (n=757) according to the type of medications used during pregnancy.[37] Despite adequate statistical power, this study has other limitations such as non-adjustment for asthma severity and other confounders as well as exposure measurement during the entire pregnancy period.[37] This 60% increased risk is not negligible since the risk of congenital malformations in the general population is believed to be about 3%.[43] The second study reported a significant increased risk of “major cardiac malformations” (aOR=2.38; 95% CI: 1.11, 5.1), “major other and unspecified malformations” (aOR=3.97; 95% CI: 1.29, 12.2) and “any genital malformations” (aOR=6.84; 95% CI: 2.58, 18.10) among asthmatic women exposed to LABA when compared to asthmatic women unexposed to LABA during the first trimester.[29] Moreover, the study found that women exposed to SABA at high doses (>10

per week) were less likely to have a baby with a major malformation. Exposure to medications was measured with prescription claims collected prospectively and independently of the outcome, avoiding recall bias. However, prescription claims might not reflect exactly the actual intake of medications.[29]

While a comparison group of asthmatic non-users of the medication under study seems theoretically appropriate, it should be recognized that asthmatic non-users might have a milder form of the disease or being under-treated, and that residual bias by indication might still be present. Confounding by indication can be reduced in an alternate way through comparing two similar therapies, i.e. two treatment regimens with the same indication. SABA and LABA cannot be included in a head-to-head comparison, but a comparison between LABA plus ICS in low or medium doses against high doses of ICS could be informative about the safety of LABA while minimizing confounding by indication, and such comparisons should be included in future studies.

Several other methodological aspects of the studies included in the review need to be considered. The source of data varied from one study to the other, being through medical charts in some, telephone and personal interviews in others, or registry records and administrative databases, and different definitions were used to assess asthma according to the source of data used. Regarding the exposure assessment, recall bias might have affected case-control studies where drug data were collected retrospectively by interviews or questionnaires causing overestimated effects. [31,32,37,40,41] On the other hand, a secondary data source does not suffer this limitation, but could be affected by non-differential misclassification that underestimates the true effects.[29,33,35,36,39] The timing of exposure during pregnancy could influence the results and lead to variability between studies.[49,50] As for congenital malformations, the most susceptible stage for the embryonic development is the first trimester, where many teratogenic agents show their effect.[49,50] On the other hand, the third trimester of pregnancy might be more relevant for other measures of fetal growth, such as mean birth weight, LBW, and SGA since the majority of the fetal growth takes place during this period.[14] For congenital malformations, twelve studies examined the use of beta$_2$-agonists during the first trimester[17,21,23,29,31,32,35,36,39-42], eight during the entire pregnancy[19-23,34,35,37] ,one during early pregnancy[38], and one did not specify when the exposure was measured.[33] For SGA, birth weight, and LBW, nine studies examined the entire pregnancy[18-24,34,39], and one had an undetermined exposure period.[33] Regarding

gestational age and preterm delivery, seven examined the entire pregnancy[18,20,22-24,34,39], and the timing of the exposure was not specified in one study.[33]

The ascertainment of outcomes varied across studies. In registry and administrative database studies, diagnostic codes were used alone to ascertain an infant outcome.[29,33,35] A potential non-differential information bias could occur if the accuracy of the information is not high enough, leading to effect measures closer to the null. Some studies used patients' interviews or hospital medical records to minimize such bias.[20-22,31,32,36,39-41]

Another source of bias is the non-response and non-participation rates which reached high levels (30% to 40%) in some studies. [31,32,37,40,41] We can argue that women exposed to beta$_2$-agonists who choose to participate in a study were more likely to have a risk factor (i.e. family history of malformation) compared to non-participants. This conditional participation could lead to biased results that over-estimate the true effects. In addition, coexisting morbidity is not uncommon among pregnant asthmatic women. Pregnant women using anti-asthmatic medications are often using other types of medications and studies reporting increased risk of congenital malformations without adjusting for concomitant drug use or maternal co-morbidities might have overestimated the effects of beta$_2$-agonists.[31,32,40,41] Moreover, some of the studies reported only crude results and several of them did not adjust for the level of asthma severity and control, which have been shown to be associated with the outcomes under study[2,4-8].

Furthermore, the negative results obtained in studies with low statistical power should be interpreted with caution because they can give a false impression of safety. Among studies investigating SABA and/or LABA use, only three among the seven studies had a power of 80% or more to detect the specified clinically significant effect. .[20,34,39] Moreover, only six studies out of the twelve investigating SABA separately [23,29,35,37,38,41] and one of the ten studies investigating LABA separately had a power of 80% or more to detect the specified effect size.[24]

This review is limited by the fact that we could not pool the different study results into a single estimate for each outcome due to major methodological differences between the studies. Another limitation of this review is that it included only studies published in English language, and excluded studies that do not have comparison groups (i.e. case-reports, case-series and PEM studies).

The strength of this review lies in the fact that we included the most relevant studies that provided information on the outcomes under study. In addition, we used the validated and recommended NOS-scale for the quality assessment of the studies. Another strength of this review is the post-hoc power calculation that we performed to identify studies able to detect clinically significant effects. Compared to previously published reviews on the effect of beta$_2$-agonist use during pregnancy [4,14-16,25,26], the spectrum of perinatal outcomes investigated in the current review was larger. Previous reviews were also limited by one or more of the following: investigating only SABA or only LABA or combining both with other bronchodilators, screening fewer databases, not assessing the quality of the studies included, and lastly providing no data on the studies' statistical power.[4,14-16,25,26]

Beta$_2$-agonists are key medications in the treatment of asthmatic pregnant women. We found a larger body of knowledge on salbutamol compared to other SABA, and that adds to the evidence of its safety. It is difficult to conclude on the safety of other SABA (i.e. fenoterol and terbutaline), so we recommend that practitioners prescribe salbutamol for pregnant women in concordance with the guidelines. Regarding LABA, there is evidence of specific congenital malformations increased risk, but it is difficult to interpret this association as causal because part of this risk might be attributable to the severity of asthma. Until this observation is reproduced in other studies, it is difficult to make a clear recommendation, and the current guidelines should be followed. Future studies should be large enough to be able to compare equivalent treatment regimens, or to compare different molecules of a class in order to minimize confounding by asthma severity and to identify the safest treatment options. Future studies might also consider meta-analysis of drug-specific effects from several well-conducted studies.

5. Conclusions

In summary, we found 21 studies that examined the effect of beta$_2$-agonists use during pregnancy on congenital malformations, fetal growth, and prematurity. We found evidence of increased risk of congenital malformations after pregnancy exposure to fenoterol (SABA) in one study[37] and LABA in another study.[29] No increased risk was found for the other outcomes, except a decrease in birth weight centiles among salmeterol (LABA) users.[24] However, non significant results should be interpreted with caution since a large percentage of the negative studies were under powered to detect clinically significant effects.

We conclude that other studies on the use of SABA and LABA during pregnancy are needed to obtain precise estimates of associated risks to rule on their safety profile.

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Contributors' statement

All the authors participated in the conception and design of the study. Mr. Eltonsy and Mrs. Kettani collected and processed the data; Mr Sherif drafted the first manuscript; Dr.Blais supervised the data processing, and all the authors read, changed, and approved the final manuscript.

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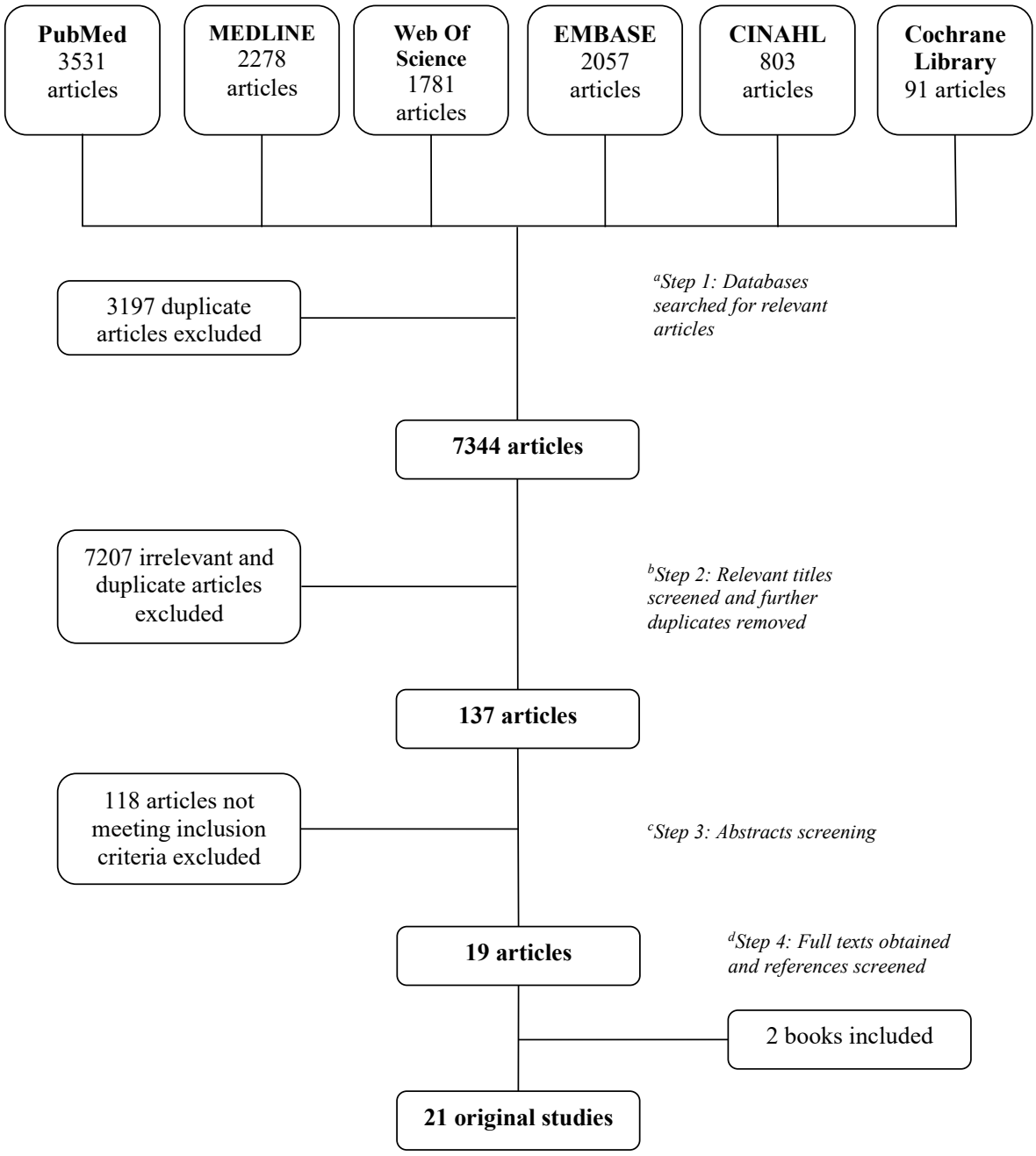

Figure 1. Strategy for the selection of the studies.

[a] Selected databases were searched for relevant articles using defined keywords. Articles retrieved from each database were imported into a separate EndNote library (version X4.0.1, Thomson Reuters). The six EndNote libraries formed from the databases were combined in one large EndNote library, and duplicates between databases were removed.

[b] Titles were screened for relevance. Studies not related to the question of interest, animal studies, review articles, and further duplicates were excluded at this step. [c] Abstracts were examined to confirm eligibility to the final selection and full text were revised. Articles only providing relevant data on beta$_2$-agonists and the pre-selected perinatal outcomes were included. Published abstracts without original articles were excluded. [d] Selected articles were obtained and data retrieved and processed. References were checked for additional articles and reviews on the topic were manually searched for further references.

Table 1. Assessment of Methodologic Quality of Studies According to the Newcastle-Ottawa Scale for Cohort and Case-control Studies

Study ref.	Country	Newcastle-Ottawa Scale			Total Score
		Selection *(max.4)*	***Comparability***[a] *(max.2)*	***Exposure/Outcome*** *(max.3)*	*(over 9 points)*
Cohort Studies					
Eltonsy et al. (2011)[29]	Canada	***	**	***	8
Clark et al. (2007)[19]	UK	*	*	***	4
Kallen et al. (2007)[36]	Sweden	**	*	***	6
Clifton et al. (2006)[24]	Australia	***		**	5
Bakhireva et al. (2005)[20]	USA	****	*	***	8
Schatz et al. (2004)[22]	USA	****	*	**	7
Bracken et al. (2003)[18]	USA	***	*	**	6
Olesen et al. (2001)[39]	Denmark	***		**	5
Alexander et al. (1998)[33]	Canada	**	*	***	6
Schatz et al. (1997)[21]	USA	**	*	***	6
Michigan Medicaid (1993)[17]	USA	**		**	4
Lao et al. (1990)[34]	Hong Kong	**		**	4
Schatz et al. (1988)[23]	USA	***	*	***	7
CPP (1977)[42]	USA	**		**	4
Case-Control Studies					
Lin et al. (2012)[41]	USA	***	*	**	6
Munsie et al. (2011)[40]	USA	***	*	**	6
Lin et al. (2009)[31]	USA	****	*	***	8
Lin et al. (2008)[32]	USA	***	*	**	6
Tata et al. (2008)[35]	UK	**	*	***	6
Tamasi et al. (2006)[37]	Hungary	***		**	5
Kallen et al. (2003)[38]	Sweden	***	*	**	6

Table 2A. Studies Investigating the Association between Beta$_2$-agonists Use during Pregnancy and Major Congenital Malformations

Study ref.	Design	Source of data	Exposure Timing	Users of ß$_2$-agonists			Non-users of ß$_2$-agonists			Effect		Power (%) for RR =1.5
				Type of ß$_2$-agonists	n*	Major congenital malformation (%)	Definition	n*	Major congenital malformation (%)	OR or RR	95% CI or (p-value)	
SABA and/or LABA use: cohort studies												
Bakhireva et al. (2005)[20]	Cohort	Tel. interviews & medical charts	Entire pregnancy	Any	103	3.9	Non asthmatics	303	0.3	cRR 13.0	NA	8
				Any	103	3.9	Asthmatics ICS users [a]	438	4.1	cRR 0.95	NA	17
Schatz et al. (2004)[22]	Cohort + RCT	Medical charts & interviews	Entire pregnancy	Any	1,828	2.0	Asthmatics non users [bcd]	295	2.0	cRR 1.0	(>0.05)	12

Study ref.	Design	Source of data	Exposure Timing	Type of ß$_2$-agonists	Cases		Controls		Definition of non-users of ß$_2$-agonists	Effect		Power (%) for RR =1.5
					Users of ß$_2$-agonists	Non-users of ß$_2$-agonists	Users of ß$_2$-agonists	Non-users of ß$_2$-agonists		OR or RR	95% CI or (p-value)	
SABA and/or LABA use: case-control studies												
Lin et al. (2012)[41]	Case Control	Registry, medical records & self reports	1 month prior conception + 1st trimester	Any [f]	10	168	NA	NA	Asthmatics and non asthmatics non users	**aOR = 2.39[o]**	**1.23, 4.66**	NC
Munsie et al. (2011)[40]	Case Control	Registry, medical records & self reports	1 month prior conception + 1st trimester	Any [f]	20	570	114	6207	Asthmatics and non-asthmatics non users	**aOR = 1.77[q]**	**1.08, 2.88**	NC
			1 month prior conception + 1st trimester	Any [f]	26	887	114	6207	Asthmatics and non-asthmatics non users	aOR = 1.53[r]	0.99, 2.37	46
			1 month prior conception + 1st trimester	Any [f]	17	1114	114	6207	Asthmatics and non-asthmatics non users	aOR = 0.78[s]	0.46, 1.31	52
			2nd & 3rd trimesters	Any [f]	7	570	58	6207	Asthmatics and non-asthmatics non users	aOR = 1.26[q]	0.57, 2.80	20
			2nd & 3rd trimesters	Any [f]	4	887	58	6207	Asthmatics and non-asthmatics non users	aOR = 0.49[r]	0.18, 1.36	24
			2nd & 3rd trimesters	Any [f]	6	1114	58	6207	Asthmatics and non-asthmatics non users	aOR = 0.59[s]	0.25, 1.38	27
Lin et al. (2009)[31]	Matched Case Control 1:2	Registry, medical records & tel. interviews	1 month prior conception + 1st trimester	Any [f]	22	443	22	965	Asthmatics and non asthmatics with no Rx	**aOR = 2.20**	**1.05,4.61**	NC

Lin et al. (2008)[32]	Case Control 1:11	Tel. interviews& Rx DB	1 month prior conception + 1st trimester	Any [g]	17	358	96	3,932	Asthmatics and non-asthmatics non users [g]	**aOR = 2.06**	**1.19,3.59**	NC

SABA only: cohort studies

Study ref.	Design	Source of data	Exposure Timing	Users of ß2-agonists			Non-users of ß2-agonists			Effect		Power (%) for RR =1.5
				Type of ß2-agonists	n*	Major congenital malformation (%)	Definition	n*	Major congenital malformation (%)	OR or RR	95% CI or (p-value)	
Eltonsy et al. (2011)[29]	Cohort	Quebec administrative DB	1st trimester	Any	7,182	5.7	Asthmatics non users[g]	5,935	5.9	aOR = 0.93	0.80,1.08	100
				Any (>0–3 doses/week)	3,420	6.1				aOR = 1.00	0.83, 1.20	99
				Any (>3–10 doses/week)	2,102	5.5				aOR = 0.84	0.67, 1.06	98
				Any (>10 doses/week)	1,660	5.2				**aOR = 0.68**	**0.48, 0.95**	NC
Kallen et al. (2007)[36]	Cohort	Swedish Registers (Medical birth, Congenital malformation and Hospital discharge)	1st trimester	Salbut	NA	NA	General population	NA	NA	**aOR = 1.38[h]**	**1.12,1.70**	NC
				Terbut	NA	NA		NA	NA	aOR = 1.08[i]	0.94,1.23	__
Schatz et al. (1997)[21]	Cohort	Daily diary cards for medications completed by patients. For outcomes, data source not precised	Entire pregnancy	Any [e]	667	3.7	Non asthmatics	823	6.2	cRR 0.60	(>0.05)	54
			1st trimester	Any [e]	488	4.3	Non asthmatics	1,000	5.6	cRR 0.77	(>0.05)	48
Schatz et al. (1988)[23]	Cohort	Questionnaire for patients identification, confirmed clinically + Self-Diary to report use of SABA & medical records (for perinatal outcomes)	Entire pregnancy	Any [bfj]	259	3.9	Non asthmatics	295	6.4	cOR = 0.61	(>0.05)	25
			1st trimester	Any [bfj]	180	3.9	Non asthmatics	295	6.4	cOR = 0.61	(>0.05)	22
			Entire pregnancy	Any [bfj]	259	3.9	Asthmatics non users[g]	101	6.0	cOR = 0.65	(>0.05)	12
			1st trimester	Any [bfj]	180	3.9	Asthmatics non users[g]	172	5.3	cOR = 0.74	(>0.05)	15
			Entire pregnancy	Any [bfj]	259	3.5	General population	1,999,254	3.0	cOR = 1.17	NA	31

SABA only: case-control studies

Study ref.	Design	Source of data	Exposure Timing	Type of ß2-agonists	Cases		Controls		Definition of non-users of ß2-agonists	Effect		Power (%) for RR =1.5
					Users of ß2-agonists	Non-users of ß2-agonists	Users of ß2-agonists	Non-users of ß2-agonists		OR or RR	95% CI or (p-value)	
Lin et al. (2012)[41]	Case Control	Registry, medical records & self reports	1 month prior conception + 1st trimester	Salbut	77	2776	139	6587	Asthmatics and non asthmatics non users	cOR = 1.31[P]	NA	81
				Pirbuterol	3	2850	3	6723	Asthmatics and non asthmatics non users	cOR = 2.36[P]	NA	8
Munsie et al. (2011)[40]	Case Control	Registry, medical records & self reports	1 month prior conception + 1st trimester	Salbut	18	570	101	6207	Asthmatics and non-asthmatics non users	**aOR = 1.79[q]**	**1.07, 2.99**	NC
			1 month prior conception + 1st trimester	Salbut	25	887	101	6207	Asthmatics and non-asthmatics non users	**aOR = 1.65[r]**	**1.06, 2.58**	NC
			1 month prior conception + 1st trimester	Salbut	15	1114	101	6207	Asthmatics and non-asthmatics non users	aOR = 0.76[s]	0.44, 1.33	48
			2nd & 3rd trimesters	Salbut	7	570	55	6207	Asthmatics and non-asthmatics non users	aOR = 1.34[q]	0.60, 2.98	22
			2nd & 3rd trimesters	Salbut	4	887	55	6207	Asthmatics and non-asthmatics non users	aOR = 0.52[r]	0.19, 1.44	27
			2nd & 3rd trimesters	Salbut	6	1114	55	6207	Asthmatics and non-asthmatics non users	aOR = 0.64[s]	0.27, 1.49	31
			1 month prior conception + 1st trimester	Pirbuterol/ Metaprot/ Epineph	4	85	5	153	Asthmatics and non asthmatics non users	cOR = 1.44	NA	9
Lin et al. (2009)[31]	Matched Case Control 1:2	Registry, medical records & tel. interviews	1 month prior conception + 1st trimester	Salbut[g]	15	443	14	965	Asthmatics and non asthmatics with no Rx	aOR = 2.37	0.90,6.23	14
				Metaprot[g]	1	31	1	42		cRR = 1.35	NA	6
				Terbut [g]	1	31	0	43		__	NA	__

Lin et al. (2008)[32]	Case Control 1:11	Tel. interviews & Rx DB	1 month prior conception + 1st trimester	Salbut/ Pirbuterol[g]	13	368	88	4,033	Asthmatics and non asthmatics non users [g]	cOR = 1.62	NA	26
Tata et al. (2008)[35]	Matched Case Control 1:6	THIN DB	Entire pregnancy	Any	375	NA	2085	NA	Asthmatics non users[bcd]	aOR = 1.06	(0.336) 0.94,1.19	100
			1st trimester		NA	NA	NA	NA	NA	aOR = 1.01	(0.941) 0.86,1.18	—

LABA only: cohort studies

Study ref.	Design	Source of data	Exposure Timing	Users of ß2-agonists			Non-users of ß2-agonists			Effect		Power (%) for RR =1.5
				Type of ß2-agonists	n*	Major congenital malformation (%)	Definition	n*	Major congenital malformation (%)	OR or RR	95% CI or (p-value)	
Eltonsy et al. (2011)[29]	Cohort	Quebec administrative DB	1st trimester	Any	165	7.9	Asthmatics non users[g]	12,952	5.8	aOR = 1.31	0.74,2.31	33
						4.2			2.0	**aOR = 2.38[k]**	**1.11,5.1**	NC
						1.8			0.5	**aOR =3.97[l]**	**1.29,12.2**	NC
Kallen et al. (2007)[36]	Cohort	Swedish Registry (Medical birth, Congenital malformation and Hospital discharge)	1st trimester	Salmeterol	NA	NA	General population	NA	NA	aOR = 1.34[m]	0.96,1.88	—
				Formoterol	NA	NA		NA	NA	aOR = 1.07[n]	0.63,1.82	—

LABA only: case-control studies

Study ref.	Design	Source of data	Exposure Timing	Type of ß2-agonists	Cases		Controls		Definition of non-users of ß2-agonists	Effect		Power (%) for RR =1.5
					Users of ß2-agonists	Non-users of ß2-agonists	Users of ß2-agonists	Non-users of ß2-agonists		OR or RR	(95% CI) or (p-value)	
Lin et al. (2012) [41]	Case Control	Registry, medical records & self reports	1 month prior conception + 1st trimester	Salmeterol	13	2840	23	6703	Asthmatics and non asthmatics non users	cOR = 1.33 [p]	NA	24
Munsie et al. (2011)[40]	Case Control	Registry, medical records & self reports	1 month prior conception + 1st trimester	Salmeterol	6	83	21	137	Asthmatics and non asthmatics non users	cOR = 0.47[s]	NA	23
Lin et al. (2008)[32]	Case Control 1:11	Tel. interviews & Rx DB	1 month prior conception + 1st trimester	Salmeterol[g]	2	379	11	4,110	Asthmatics and non asthmatics non users [g]	cOR = 1.97	NA	7
Tata et al. (2008)[35]	Matched Case Control 1:6	THIN DB	Entire pregnancy	Any	25	NA	131	NA	Asthmatics non users[bcd]	aOR = 1.12	(0.614) 0.72,1.75	49
			1st trimester		NA	NA	NA	NA		aOR = 1.09	(0.77) 0.62,1.9	—

Table 2B. Studies Investigating the Association between Beta$_2$-agonists Use during Pregnancy and Any Congenital Malformations

Study ref.	Design	Source of data	Exposure Timing	Users of ß$_2$-agonists			Non-users of ß$_2$-agonists			Effect		Power (%) for RR =1.5
				Type of ß$_2$-agonists	n*	All congenital malformations (%)	Definition	n*	All congenital malformations (%)	OR or RR	95% CI or (p-value)	
SABA and/or LABA use: cohort studies												
Clark et al. (2007)[19]	Cohort	Questionnaire & medical charts	Entire pregnancy	Any	178	2.2	Non asthmatics	717	2.2	cRR = 1.0 [a]	NA	16
				Any	178	2.2	Asthmatics with no Rx	370	0.8	cRR = 2.75[a]	NA	9
Olesen et al. (2001)[39]	Cohort	Registry & Rx DB	1 month prior until 8 weeks of pregnancy	Any	272	NA	Non users of any Rx [b]	8,717	NA	NA	(<0.05)	__
Alexander et al. (1998)[33]	Cohort	Registry (perinatal DB) & medical charts	Not determined	Any	303	8.5	Non asthmatics	13,709	7.7	aOR = 1.0	0.6,1.6	58
				Any	303	8.5	Asthmatics with no Rx	375	6.9	aOR = 0.9	0.6,1.4	31
				Any	303	8.5	Asthmatics steroid users [c]	139	6.2	aOR = 0.8	0.4,1.7	16
Lao et al. (1990)[34]	Cohort	Hospitals DB	Entire pregnancy	Any[de]	54	3.8	Non asthmatics	54	0.0	__	NA	__
SABA only: cohort studies												
Eltonsy et al. (2011)[29]	Cohort	Quebec administrative DB (medical services and Rx DB)	1st trimester	Any	7,182	9.6	Asthmatics non users[c]	5,935	9.3	aOR = 1.04	0.92,1.17	100
				Any (>0–3 doses/week)	3,420	10.0				aOR = 1.08	0.94, 1.25	100
				Any (>3–10 doses/week)	2,102	9.9				aOR = 1.07	0.90, 1.26	99
				Any (>10 doses/week)	1,660	8.5				aOR = 0.90	0.74, 1.09	99
Kallen et al. (2007)[36]	Cohort	Swedish Registers (Medical birth, Congenital malformation and Hospital discharge)	1st trimester (early pregnancy, usually 10-12 weeks)	Salbut	NA	NA	General population	NA	NA	aOR = 1.09	0.97,1.75	__
				Terbut	NA	NA		NA	NA	**aOR = 1.11**	**1.04,1.19**	NC
Michigan Medicaid (1993)[17]	Cohort	Surveillance registry	1st trimester	Isoprot	16	6.3[f]	NA	NA	NA	cRR = 1.4	NA	__
				Salbut	1,090	4.4[f]	NA	NA	NA	cRR = 1.1	NA	__
				Terbut	149	4.7[f]	NA	NA	NA	cRR = 1.2	NA	__
				Metaprot	361	4.7[f]	NA	NA	NA	cRR = 1.1	NA	__
				Isoetharine	22	0.0[f]	NA	NA	NA	__	NA	__
				Epineph	35	0.0[f]	NA	NA	NA	__	NA	__
CPP (1977)[42]	Cohort	Surveillance registry	Early pregnancy (1st trimester)	Isoprot	31	NA	NA	NA	NA	cRR = 0.9	NA	__
				Ephed	373	NA	NA	NA	NA	cRR = 1.1	NA	__
				Epineph	189	NA	NA	NA	NA	**cRR = 1.7**	**(<0.05)**	NC

SABA only: case-control studies

Study ref.	Design	Source of data	Exposure Timing	Type of ß2-agonists	Cases		Controls		Definition of non-users of ß2-agonists	Effect		Power (%) for RR =1.5
					Users of ß2-agonists	Non-users of ß2-agonists	Users of ß2-agonists	Non-users of ß2-agonists		OR or RR	95% CI or (p-value)	
Tamasi et al. (2006)[37]	Matched Case control 1:3	Hungarian registry & questionnaire (self-administered) + medical records	Entire pregnancy	Salbut	45	466	77	680	Asthmatics non users	cOR = 0.9	0.6, 1.3	57
				Terbut	179	332	241	516		cOR = 1.2	0.9, 1.5	92
				Metaprot	3	508	6	751		cOR = 0.7	0.2, 3.0	10
				Fenoterol	328	183	403	354		**cOR = 1.6**	**1.3, 2.0**	NC
Kallen et al. (2003)[38]	Case control	Swedish Medical birth Register & Interview	Early pregnancy	Salbut	29	4,986	3,446	574,284	Asthmatics and non asthmatics non users	cOR = 0.97 aOR = 0.93	0.64, 1.36	70
				Terbut	104	4,911	10,613	567,117		cOR = 1.13 aOR = 1.14	0.93, 1.38	98

LABA only: cohort studies

Study ref.	Design	Source of data	Exposure Timing	Users of ß2-agonists			Non-users of ß2-agonists			Effect		Power (%) for RR =1.5
				Type of ß2-agonists	n*	All congenital malformations (%)	Definition	n*	All congenital malformations (%)	OR or RR	95% CI or (p-value)	
Eltonsy et al. (2011)[29]	Cohort	Quebec administrative DB (medical services and Rx DB)	1st trimester	Any	165	12.7	Asthmatics non users[g]	12,952	9.4	aOR = 1.37	0.92,2.17	44
						3.0			0.7	**aOR = 6.84**[g]	**2.58,18.10**	NC
						3.0			0.9	**aOR=3.43**[h]	**1.39,8.45**	NC
Kallen et al. (2007)[36]	Cohort	Swedish Registry (Medical birth, Congenital malformation and Hospital discharge)	1st trimester (early pregnancy, usually 10-12 weeks)	Salmeterol	NA	NA	General population	NA	NA	aOR = 1.02	0.83,1.25	__
				Formoterol	NA	NA		NA	NA	aOR = 1.06	0.80,1.40	__

LABA only: case-control studies

Study ref.	Design	Source of data	Exposure Timing	Type of ß2-agonists	Cases		Controls		Definition of non-users of ß2-agonists	Effect		Power (%) for RR =1.5
					Users of ß2-agonists	Non-users of ß2-agonists	Users of ß2-agonists	Non-users of ß2-agonists		OR or RR	95% CI or (p-value)	
Tamasi et al. (2006)[37]	Matched case control 1:3	Hungarian registry & questionnaire (self-administered) + medical records	Entire pregnancy	Clenbuterol	28	483	56	701	Asthmatics non users	cOR = 0.7	0.5, 1.2	57

Kallen et al. (2003)[38]	Case control	Swedish Medical birth Registry & Interview	Early pregnancy	Salmeterol	15	5,000	1,137	576,593	Asthmatics and non asthmatics non users	aOR = 1.50	0.90, 2.53	35

Table 3. Studies Investigating the Association between Beta$_2$-agonists Use during Pregnancy and SGA

Study ref.	Design	Source of data	Exposure Timing	Users of ß$_2$-agonists			Non-users of ß$_2$-agonists			Effect		Power (%) for RR =1.5
				Type of ß$_2$-agonists	n*	SGA (%)	Definition	n*	SGA (%)	OR or RR	95% CI or (p-value)	
SABA and/or LABA use: cohort studies												
Clark et al. (2007)[19]	Cohort	Questionnaire & medical charts	Entire pregnancy	Any	99 boys	22.2	Non asthmatics	370 boys	27.8	cRR = 0.80[a]	NA	40
								347 girls	28.8	cRR = 0.92[a]	NA	35
							Asthmatics with no Rx	191 boys	29.3	cRR = 0.76[a]	NA	35
					79 girls	26.6		179 girls	22.9	cRR = 1.16[a]	NA	28
Bakhireva et al. (2005)[20]	Cohort	Tel.interviews & medical charts	Entire pregnancy	Any	103	3.9	Non asthmatics	303	5.0	cRR = 0.78 aOR = 0.57	(>0.05) 0.16,2.12	17
				Any	103	3.9	Asthmatics ICS users [b]	438	6.2	cRR = 0.63 aOR = 0.50	0.13, 1.89	20
Schatz et al. (2004)[22]	Cohort + RCT	Medical charts & interviews	Entire pregnancy	Any	1,828	7.1	Asthmatics non users [cde]	295	7.2	cRR = 0.99	(>0.05)	39
SABA only: cohort studies												
Bracken et al. (2003)[18]	Cohort	Tel. interviews + medical charts	Entire pregnancy	Any	401	7.5	Asthmatics and non asthmatics non users[f]	1,800	7.7	cOR = 0.97[g] aOR = 1.0[h]	0.65,1.47[g] 0.99,1.01[h]	60
Schatz et al. (1997)[21]	Cohort	Daily diary cards for medications completed by patients. For outcomes, data source not precised	Entire pregnancy	Any [i]	NA	NA	Non asthmatics	NA	NA	NA	(>0.05)	__
Schatz et al. (1988)[23]	Cohort	Questionnaire for patients identification, confirmed clinically + Self-diary to report use of SABA & medical records (for perinatal outcomes)	Entire pregnancy	Any[cjk]	259	1.6	Non asthmatics	295	1.0	cRR = 1.6	(>0.05)	9
							Asthmatics non users [f]	101	3.1	cRR = 0.52	(>0.05)	8
LABA only: cohort studies												
Clifton et al. (2006)[24]	Cohort	Medical charts	Entire pregnancy	Salmeterol[l]	9	22.2	Non asthmatics	20	10.0	cRR = 2.2	(>0.05)	9
							Asthmatics fluticasone users	18	11.1	cRR = 2.0	(>0.05)	8
							Asthmatics budesonide users	14	0	__	NA	__
Bracken et al. (2003)[18]	Cohort	Tel. interviews + medical charts	Entire pregnancy	Any	48	8.3	Asthmatics and non asthmatics non users [f]	2,153	7.6	cOR = 1.1[g] aOR = 1.0[h]	0.39,3.11[g] 0.99,1.02[h]	18

Table 4A. Studies Investigating the Association between Beta$_2$-agonists Use during Pregnancy and Birth Weight

Study ref.	Design	Source of data	Exposure Timing	Users of ß$_2$-agonists			Non-users of ß$_2$-agonists			Effect		Power (%) for MD = 500g
				Type of ß$_2$-agonists	n*	Mean birth weight in g (SD)	Definition	n*	Mean birth weight in g (SD)	MD in g	95% CI or (p-value)	
SABA and/or LABA use: cohort studies												
Clark et al. (2007)[19]	Cohort	Questionnaire & medical charts	Entire pregnancy	Any	99 boys	3545[a]	Non asthmatics	370 boys	3320[a]	cMD = 225 [b]	NA	—
								347 girls	3240[a]	cMD = -20 [b]	NA	—
					79 girls	3220[a]	Asthmatics with no Rx	191 boys	3360[a]	cMD = 185 [b]	NA	—
								179 girls	3260[a]	cMD = -40 [b]	NA	—
Bakhireva et al. (2005)[20]	Cohort	Tel. interviews & medical charts	Entire pregnancy	Any	103	3,552 (51)	Non asthmatics	303	3,540 (29)	aMD = 12	(>0.05)	100
				Any	103	3,552 (51)	Asthmatics ICS users [c]	438	3,524 (24)	aMD = 28	(>0.05)	100
Olesen et al. (2001)[39]	Cohort	Registry & Rx DB	Entire pregnancy	Any	272	3,361.5 (571.3)	Non users of any Rx [d]	8717	3,414 (579)	aMD = -45.8	-115.1 ,23.5	100
Lao et al. (1990)[34]	Cohort	Hospitals DB	Entire pregnancy	Any [ef]	54	3,226 (453)	Non asthmatics	54	3,281 (328)	cMD = -55	(>0.05)	100
SABA only: cohort studies												
Schatz et al. (1988)[23]	Cohort	Questionnaire for patients identification, confirmed clinically + Self-Diary to report use of SABA & medical records (for perinatal outcomes)	Entire pregnancy	Any [egh]	259	3,416 (35)[i]	Non asthmatics	295	3,477 (32) [i]	cMD = -61	(>0.05)	100
							Asthmatics non users [g]	101	3,361 (68) [i]	cMD = 55	(>0.05)	100
LABA only: cohort studies												
Clifton et al. (2006)[24]	Cohort	Medical charts	Entire pregnancy	Salmeterol[j]	9	3,283 (120.3)[i]	Non asthmatics	20	3,423.3 (122)	cMD = -140.3	(>0.05)	100
							Asthmatics fluticasone users	18	3,441.7 (149.4)	cMD = -158.7	(>0.05)	100
							Asthmatics budesonide users	14	3,824.6 (100)	cMD = -541.6	(>0.05)	100
						34.8 centile (9.3)	Non asthmatics	20	47.7 (7.4)	cMD = -12.9	(>0.05)	75[k]
							Asthmatics fluticasone users	18	53.6 (7.1)	cMD = -18.8	(>0.05)	75[k]
							Asthmatics budesonide users	14	74.0 (5.4)	**cMD = -39.2**	**(0.011)**	NC

Table 4B. Studies Investigating the Association between Beta$_2$-agonists Use during Pregnancy and Low Birth Weight (< 2500 g)

Study ref.	Design	Source of data	Exposure Timing	Users of ß$_2$-agonists			Non-users of ß$_2$-agonists			Effect		Power (%) for RR =1.5
				Type of ß$_2$-agonists	n*	Low birth weight (%)	Definition	n*	Low birth weight (%)	OR or RR	95% CI or (p-value)	
SABA and/or LABA use: cohort studies												
Schatz et al. (2004)[22]	Cohort + RCT	Medical charts & interviews	Entire pregnancy	Any	1,828	13.5	Asthmatics non-users [abc]	295	15.2	cRR = 0.89	(>0.05)	67
Alexander et al. (1998)[33]	Cohort	Registry (perinatal DB) & medical charts	Not determined	Any	303	7.9	Non asthmatics	13,709	5.6	aOR = 1.4	0.8,2.2	49
				Any	303	7.9	Asthmatics with no Rx	375	4.9	aOR = 0.9	0.5,1.5	24
				Any	303	7.9	Asthmatic steroid users[d]	139	5.1	aOR = 1.0	0.4,2.5	13
Lao et al. (1990)[34]	Cohort	Hospitals DB	Entire pregnancy	Any [a]	54	5.6	Non asthmatics	54	1.9	cRR = 2.9	(>0.05)	3
SABA only: cohort studies												
Schatz et al. (1997)[21]	Cohort	Daily diary cards for medications completed by patients. For outcomes, data source not precised	Entire pregnancy	Any[e]	NA	NA	Non asthmatics	NA	NA	NA	(>0.05)	—
Schatz et al. (1988)[23]	Cohort	Questionnaire for patients identification, confirmed clinically + Self-Diary to report use of SABA & medical records (for perinatal outcomes)	Entire pregnancy	Any[afg]	259	4.6	Non asthmatics	295	3.1	cRR = 1.48	(>0.05)	15
							Asthmatics non users[d]	101	6.0	cRR = 0.77	(>0.05)	12

Table 5A. Studies Investigating the Association between Beta$_2$-agonists Use during Pregnancy and Gestational Age (weeks)

Study ref.	Design	Source of data	Exposure Timing	Users of ß$_2$-agonists			Non-users of ß$_2$-agonists			Effect		Power (%) for MD = 1 week
				Type of ß$_2$-agonists	n*	Gestational age in weeks (SD)	Definition	n*	Gestational age in weeks (SD)	MD	95% CI or (p-value)	
SABA and/or LABA use: cohort studies												
Bakhireva et al. (2005)[20]	Cohort	Tel. interviews & medical charts	Entire pregnancy	Any	103	39.4 (1.8)	Non asthmatics	303	39.2 (1.5)	cMD = 0.2	(>0.05)	100
				Any	103	39.4 (1.8)	Asthmatics ICS users[a]	438	39.4 (1.8)	cMD = 0	(>0.05)	100
Olesen et al. (2001)[39]	Cohort	Registry & Rx DB	Entire pregnancy	Any	272	276.2 (15 days)	Non users of any Rx[b]	8717	276.1 (14.5 days)	aMD = -0.2	-2.0, 1.5	100
Lao et al. (1990)[34]	Cohort	Hospitals DB	Entire pregnancy	Any[c]	54	39.3 (1.7)	Non asthmatics	54	39.2 (1.5)	cMD = 0.1	(>0.05)	90
LABA only: cohort studies												
Clifton et al. (2006)[24]	Cohort	Medical charts	Entire pregnancy	Salmeterol[d]	9	39.9 (0.4)[e]	Non asthmatics	20	40.2 (0.3)[e]	cMD = -0.3	(>0.05)	100
							Asthmatics fluticasone users	18	39.6 (0.3)[e]	cMD = 0.3	(>0.05)	100
							Asthmatics budesonide users	14	39.7 (0.3)[e]	cMD = 0.2	(>0.05)	100

Table 5B. Studies Investigating the Association between Beta$_2$-agonists Use during Pregnancy and Preterm delivery (<37 weeks)

Study ref.	Design	Source of data	Exposure Timing	Users of ß$_2$-agonists			Non-users of ß$_2$-agonists			Effect		Power (%) for RR =1.5
				Type of ß$_2$-agonists	n*	Preterm delivery (%)	Definition	n*	Preterm delivery (%)	OR or RR	95% CI or (p-value)	
SABA and/or LABA use: cohort studies												
Schatz et al. (2004)[22]	Cohort + RCT	Medical charts & interviews	Entire pregnancy	Any	1828	15.8	Asthmatics non users [abc]	295	19.3	cRR = 0.82	(>0.05)	76
Alexander et al. (1998)[33]	Cohort	Registry (perinatal DB) & medical charts	Not determined	Any	303	6.0	Non asthmatics	13,709	6.0	aRR = 1.0	0.5,1.8	51
				Any	303	6.0	Asthmatics with no Rx	375	5.6	aRR = 1.0	(0.5,1.7)	27
				Any	303	6.0	Asthmatic steroid users [d]	139	7.7	aOR = 1.4	0.6, 3.0	18
Lao et al. (1990)[34]	Cohort	Hospitals DB	Entire pregnancy	Any[a]	54	1.9	Non asthmatics	54	0	__	NA	__
SABA only: cohort studies												
Bracken et al. (2003)[18]	Cohort	Tel. interviews & medical charts	Entire pregnancy	Any	529	7.6	Asthmatics and non asthmatics non users[d]	1,676	6.7	cOR = 1.14[e] aOR = 1.01[f]	0.79,1.66[e] 1.00,1.02[f]	63
Schatz et al. (1988)[23]	Cohort	Questionnaire for patients identification, confirmed clinically + Self-Diary to report use of SABA & medical records (for perinatal outcomes)	Entire pregnancy	Any [agh]	259	3.9	Non asthmatics	295	2.7	cRR = 1.44	(>0.05)	15
							Asthmatics non users [d]	101	6.0	cRR = 0.65	(>0.05)	12
LABA only: cohort studies												
Bracken et al. (2003)[18]	Cohort	Tel. interviews & medical charts	Entire pregnancy	Any	64	10.9	Asthmatics and non asthmatics non users[d]	2,141	6.8	cOR = 1.69 [e] aOR = 0.99 [f]	0.76,3.77 [e] 0.97,1.02 [f]	20

Footnotes

Table 1

[a] In rating comparability of groups, we awarded a study one star if it controlled for asthma severity/control, and another star if it controlled for other relevant confounders.

Table 2 A

Women participating in the different studies were asthmatic unless stated otherwise.
***** Number of pregnancies unless stated otherwise.
[a] Women may have concurrently received short-acting beta$_2$-agonists (inhaled or systemic).
[b] Women may have concurrently received inhaled corticosteroids.
[c] Women may have concurrently received asthma controller medications (leukotriene modifiers).
[d] Women may have concurrently received systemic corticosteroids (oral or intravenous).
[e] Women may have received inhaled, oral or injectable beta$_2$-agonists.
[f] Women may have concurrently received asthma controller medications (theophylline or ipratropium).
[g] Women may have concurrently received any other type of asthma medication.
[h] The OR presented for the association between salbutamol and cardiac malformations (92 cases reported)
[i] The OR presented for the association between terbutaline and cardiac malformations (228 cases reported)
[j] Women may have concurrently received asthma controller medications (cromolyn).
[k] The OR presented for the association between LABA and major cardiac malformations
[l] The OR presented for the association between LABA and major "other and unspecified malformations"
[m] The OR presented for the association between salmeterol and cardiac malformations (35 cases reported)
[n] The OR presented for the association between formoterol and cardiac malformations (14 cases reported)
[o] The OR presented for the association between beta$_2$-agonists and esophageal atresia
[p] The OR presented for the association between beta$_2$-agonists and selected defects including diaphragmatic hernia, esophageal atresia, small intestinal atresia, anorectal atresia, neural tube defects, omphalocele, or limb deficiencies with no additional major defect (isolated).
[q] The OR presented for the association between beta$_2$-agonists and cleft lip only
[r] The OR presented for the association between beta$_2$-agonists and cleft palate only
[s] The OR presented for the association between beta$_2$-agonists and cleft lip with cleft palate

DB: database; ICS: inhaled corticosteroids; Salbut: Salbutamol; Isoprot: Isoproterenol; Metaprot: Metaproterenol; Terbut: Terbutaline; Epineph: Epinephrine; Ephed: Ephedrine; SABA: Short acting beta$_2$-agonists; LABA: Long acting beta$_2$-agonists; RCT: randomized controlled trial; THIN: Health Improvement Network primary care database, Rx: prescription medications; aOR: adjusted odds ratio; cOR: crude odds ratio; cRR: crude risk ratio; cMD: crude mean difference; aMD: adjusted mean difference; pOR :crude prevalence odds ratio; NA: data unavailable; – : power or effect size impossible to calculate; NC: statistical power not calculated since results are significant.

Table 2 B

Women participating in the different studies were asthmatic unless stated otherwise.
***** Number of pregnancies unless stated otherwise.
[a] Matching was performed and not considered in crude calculations.
[b] Reference group formed from women who did not purchase any prescription drugs during pregnancy.
[c] Women may have concurrently received any other type of asthma medication.
[d] Women may have concurrently received inhaled corticosteroids.
[e] Women may have concurrently received oral asthma medication.
[f] Expected cases of malformations were: 0.7 case with isoproterenol, 43 with salbutamol, 6 with terbutaline, 15 with metaproterenol, 1 with isoetharine, and 1 with epinephrine.
[g] The OR presented for the association between LABA and any genital malformations
[h] The OR presented for the association between LABA and any "other and unspecified malformations"

Table 3

Women participating in the different studies were asthmatic unless stated otherwise.
***** Number of pregnancies unless stated otherwise.
[a] Matching was performed and not considered in crude calculations.
[b] Women may have concurrently received short-acting beta$_2$-agonists (inhaled or systemic).
[c] Women may have concurrently received inhaled corticosteroids.
[d] Women may have concurrently received asthma controller medications (leukotriene modifiers).
[e] Women may have concurrently received systemic corticosteroids (oral or intravenous).
[f] Women may have concurrently received any other type of asthma medication.
[g] Exposure to medication: yes/no.
[h] Exposure to medication: average doses per month.
[i] Women may have received inhaled, oral or injectable beta$_2$-agonists.
[j] Women may have concurrently received asthma controller medications (theophylline or ipratropium).
[k] Women may have concurrently received asthma controller medications (cromolyn).
[l] Salmeterol was used in combination with fluticasone propionate

Table 4 A

Women participating in the different studies were asthmatic unless stated otherwise.
***** Number of pregnancies unless stated otherwise.
[a] Median is presented (data on means are unavailable)
[b] Matching was performed and not considered in crude calculations.
[c] Women may have concurrently received short-acting beta$_2$-agonists (inhaled or systemic).
[d] Reference group formed from women who did not purchase any prescription drugs during pregnancy.

[e] Women may have concurrently received inhaled corticosteroids.
[f] Women may have concurrently received oral asthma medication.
[g] Women may have concurrently received asthma controller medications (theophylline or ipratropium).
[h] Women may have concurrently received asthma controller medications (cromolyn).
[i] Standard errors of measurements (SEM) are given in parentheses
[j] Salmeterol was used in combination with fluticasone propionate
[k] Power calculated for a difference in the mean birth weight centile =10

Table 4 B

Women participating in the different studies were asthmatic unless stated otherwise.
***** Number of pregnancies unless stated otherwise.
[a] Women may have concurrently received inhaled corticosteroids.
[b] Women may have concurrently received asthma controller medications (leukotriene modifiers).
[c] Women may have concurrently received systemic corticosteroids (oral or intravenous).
[d] Women may have concurrently received any other type of asthma medication.
[e] Women may have received inhaled, oral or injectable $beta_2$-agonists.
[f] Women may have concurrently received asthma controller medications (theophylline or ipratropium).
[g] Women may have concurrently received asthma controller medications (cromolyn).

Table 5 A

Women participating in the different studies were asthmatic unless stated otherwise.
***** Number of pregnancies unless stated otherwise.
[a] Women may have concurrently received short-acting $beta_2$-agonists (inhaled or systemic).
[b] Reference group formed from women who did not purchase any prescription drugs during pregnancy.
[c] Women may have concurrently received inhaled corticosteroids.
[d] Salmeterol was used in combination with fluticasone propionate
[e] Standard errors of measurements (SEM) are given in parentheses

Table 5 B

Women participating in the different studies were asthmatic unless stated otherwise.
***** Number of pregnancies unless stated otherwise.
[a] Women may have concurrently received inhaled corticosteroids.
[b] Women may have concurrently received asthma controller medications (leukotriene modifiers).
[c] Women may have concurrently received systemic corticosteroids (oral or intravenous).
[d] Women may have concurrently received any other type of asthma medication.
[e] Exposure to medication: yes/no.
[f] Exposure to medication: average doses per month.

[g] Women may have concurrently received asthma controller medications (theophylline or ipratropium).
[h] Women may have concurrently received asthma controller medications (cromolyn).

5.2 Article on LABA-ICS combination versus ICS monotherapy in higher doses

Risk of congenital malformations for asthmatic pregnant women using a long-acting β_2-agonist and inhaled corticosteroid combination versus higher-dose inhaled corticosteroid monotherapy

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Risk of congenital malformations for asthmatic pregnant women using a long-acting β_2-agonist and inhaled corticosteroid combination versus higher-dose inhaled corticosteroid monotherapy

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Clinical implications

Findings support the fetal safety of LABA/ICS combination in the management of persistent asthma during pregnancy, encouraging clinicians to prescribe either combination or ICS monotherapy to keep asthma under control.

Capsule summary

This comparative safety study examined the major malformations prevalence of two widely used treatment options for persistent asthma during pregnancy. Clinicians are offered new evidence on the fetal safety of LABA/ICS combination and ICS monotherapy.

Keywords: Asthma, pregnancy, congenital malformations, inhaled corticosteroid, long-acting beta2-agonist, combination therapy, high dose ICS, cohort study, comparative safety study, administrative health databases.

Abbreviations: LABA: long-acting beta$_2$-agonists, ICS: inhaled corticosteroids, GEE: generalized estimating equation , RAMQ*: Régie de l'assurance-maladie du Québec*, MED-ECHO: *Maintenance et Exploitation des Données pour l'Étude de la Clientèle Hospitalière*, PPV : predictive positive value , PNV : predictive negative value.

ABSTRACT

Background: Current recommendations for managing persistent asthma during pregnancy when low-dose inhaled corticosteroids (ICSs) are insufficient include adding a long-acting beta$_2$-agonist (LABA) or increasing the ICS dose. However, there are no data to help clinicians evaluate the safest regimen during pregnancy. **Objective**: We sought to compare the risk of major congenital malformations in asthmatic women exposed to a LABA plus ICS combination and those exposed to ICS monotherapy at higher doses during the first trimester. **Methods**: A cohort of asthmatic pregnant women exposed to ICSs during the first trimester who delivered between January 1990 and March 2009 was established. The primary outcome was major malformation recorded at birth or during the first year of life. Two subcohorts were established as follows: (1) users of a LABA plus low-dose ICS combination or users of a medium-dose ICS and (2) users of a LABA plus medium-dose ICS combination or users of a high-dose ICS. Generalized estimating equations were used to compare the risk of major malformations between the groups. **Results**: In one subcohort there were 643 women who used a LABA plus low-dose ICS and 305 who used a medium-dose ICS; the other subcohort included 198 users of a LABA plus medium dose ICS and 156 users of a high-dose ICS. The prevalence of major malformations was 6.9% and 7.2%, respectively. The adjusted odds ratio for major malformations was 1.1 (95% CI, 0.6-1.9) when a LABA plus low-dose ICS was used compared with a medium-dose ICS and 1.2 (95% CI, 0.5-2.7) when a LABA plus medium-dose ICS was used compared with a high-dose ICS. **Conclusion**: The risk of major malformations was similar with a LABA plus ICS combination and ICS monotherapy at higher doses, suggesting that both therapeutic options can be considered during pregnancy

INTRODUCTION

Asthma is one of the most common serious diseases among women of childbearing age, affecting 4%–12% of pregnant women.(1-5) Moreover, pregnant women with severe or uncontrolled asthma are at higher risk of pregnancy complications and adverse fetal outcomes than women with controlled asthma.(4, 6-8) Consequently, asthma management guidelines recommend the active treatment of asthma with appropriate medications during pregnancy to prevent asthma symptoms and exacerbation.(4, 9, 10)

Asthma management during pregnancy is based on a stepwise approach that requires an initial assessment of the level of severity and subsequent evaluations of its control.(4, 11) When asthma cannot be controlled with a low dose of inhaled corticosteroid (ICS), the controller therapy options preferred by the guidelines are either the addition of a long-acting inhaled beta$_2$-agonist (LABA) to low-dose ICS or increasing the dose of ICS to the medium-dose range. Similarly, for women with more severe asthma that is not controlled with a medium dose of ICS, the guidelines recommend the addition of a LABA to the medium-dose ICS or increasing the ICS to the high-dose range.(4, 9)

However, there has been no direct comparison of these treatment regimens to guide physicians in whether it is safer to increase the dose of ICS during pregnancy or to add a LABA. The current literature reports increasing evidence of the safety of low-to-medium doses of ICS during pregnancy (compared with no use), but indicate a possible increased risk of congenital malformations with high doses of ICS during pregnancy. There is also evidence that the ICS/LABA combination is superior to an increased dose of ICS in nonpregnant adults.(9, 12) In contrast, only limited observational data are available on the safety of LABA during pregnancy,(13) and a recently published study by our group reported a significantly increased risk of major cardiac malformations in women exposed to LABA during the first trimester.(14)

Patients' and physicians' perceptions of the teratogenicity of a medication could influence their decisions to continue or change the treatment regimen during pregnancy.(15-17) Among the important clinical decisions that physicians must make if asthma cannot be controlled with a low dose of ICS during pregnancy is whether to prescribe LABA to supplement the current dose of ICS or to increase the dose of ICS.

Evidence for the fetal safety of each treatment option is required if an informed decision is to be made. In this study, we compared the risk of major congenital malformations in pregnant asthmatic women treated with a combination of LABA and ICS and those treated with a higher dose of ICS monotherapy.

METHODS

Sources of the data

The data analyzed in this study were retrieved from the *Régie de l'assurance-maladie du Québec* (RAMQ) database and the *Maintenance et Exploitation des Données pour l'Étude de la Clientèle Hospitalière* (MED-ECHO) database. The RAMQ database contains data on the medical services provided to all residents of Quebec and data on the prescription medications dispensed in community pharmacies for residents covered by the RAMQ's Public Drug Insurance Plan (around 42% of the residents of Quebec). The MED-ECHO database contains data on acute-care hospitalization and covers all the residents of Quebec. The validity of the diagnoses of asthma and congenital malformations recorded in the RAMQ and MED-ECHO databases has been formally evaluated and the data were shown to have a predictive positive value (PPV) of 75% and a predictive negative value (PNV) of 96% for asthma diagnoses, and 82% and 88%, respectively, for diagnoses of congenital malformation.(18, 19) The prescription data recorded in the RAMQ database have been formally evaluated and found to be accurate and valid (83% correct identification of the patients and drugs dispensed from the prescriptions).(20)

Study design

To achieve our objective, a population-based retrospective cohort design was used. The cohort was selected from the *Quebec Asthma and Pregnancy Database*, which includes all pregnancies in asthmatic women and a random sample of pregnancies in nonasthmatic women between January 1, 1990 and March 31, 2010, identified from the hospitalization for delivery records in the MED-ECHO database. Using gestational age at birth and the date of birth of the newborns, we retrospectively identified the date of the

first day of the last menstrual period and the date of delivery for each pregnancy using a validated algorithm.(21) The cohort inclusion criteria were: (1) a pregnancy with a recorded singleton delivery between January 1, 1990 and March 31, 2009, so that at least one year of follow-up data was available for the newborn; (2) at least one asthma diagnosis in the two years preceding delivery (International Classification of Diseases [ICD] ICD-9 code 493 [except 493.2], or ICD-10 code J45); (3) the use of ICS in the first trimester of pregnancy (1–14 weeks); and (4) coverage with the RAMQ's Public Drug Insurance Plan for at least three months before and throughout pregnancy. The exclusion criteria were: (1) multiple births from a single pregnancy (2) rare maternal condition affecting fetal development (rheumatic disease, Cushing disease, iodine deficiency, adrenal tumor, and folic acid deficiency);(22) (3) fetal infection;(22) (4) at least one filled prescription for a teratogenic medication in the first trimester;(23, 24) (5) chronic use of an oral corticosteroid (OCS) in the first trimester (i.e., ≥ 30 days' supply); and (6) at least one filled prescription for an oral beta$_2$-agonist, leukotriene-receptor antagonist, theophylline, ipratropium, cromoglycate, or nedocromil in the first trimester. For women contributing more than one pregnancy during the study period, we included only the two most recent pregnancies to allow converging regression models. This article reports the first results for congenital malformations derived from the *Quebec Asthma and Pregnancy Database.*

Congenital malformations

Cases of major congenital malformations were identified using the ICD-9/ICD-10 hospital-based diagnostic codes recorded in the RAMQ or MED-ECHO databases at birth or during the first year of life of the infant. The codes used were specific to congenital malformations (ICD-9: 740–759, and ICD-10: Q00–Q99) and our list of malformations was compared with the list provided by the Collaborative Perinatal Group and their exactness and completeness verified by a geneticist from *le Centre Hospitalier Universitaire Sainte-Justine* in Montreal.(25) A congenital malformation was defined as major if it was life threatening or could cause major cosmetic defects. When a malformation could be classified as major or minor by the geneticist, we considered it as major only if there was at least one hospitalization with a primary diagnosis or admission

diagnosis related to this malformation that was recorded in the MED-ECHO database during the first year of life of the newborn. The specific major malformation classes and their related diagnostic codes are presented in Table E1. The primary outcome was any major congenital malformation.

Subcohorts and assessment of exposure

LABA use (salmeterol or formoterol) was defined as filling at least one prescription during the first trimester or three months before pregnancy, with the likelihood of its use during the first trimester based on the date and duration of the filled prescription (the algorithm used is available upon request). For ICS exposure (fluticasone, beclomethasone, triamcinolone, flunisolide, budesonide, or ciclesonide), we estimated the average daily dose taken during the first trimester. The estimate was made using an algorithm that we developed for previous studies, which is based upon the name of the medication, the equivalence between the different ICS products recognized by the Canadian Asthma Consensus Guidelines (in fluticasone equivalents),(26) the dose prescribed, the date and duration of the filled prescription, and the rate of renewal of the prescription.(27, 28) The daily dose of ICS was categorized as follow: low dose (> 0–250 μg), medium dose (> 250–500 μg), and high dose (> 500 μg). These algorithms accounted for the combination therapy (LABA plus ICS) being administered with a fixed-combination inhaler (salmeterol/fluticasone or formoterol/budesonide) or with separate inhalers. Two subcohorts were established to compare the treatment regimens indicated for women with similar levels of asthma severity. In the first subcohort (hereafter referred to as the "moderate asthma subcohort"), we compared women who used LABA plus low-dose ICS with those who used a medium-dose ICS monotherapy. In the second subcohort (hereafter referred to as the "severe asthma subcohort"), we compared women who used LABA plus medium-dose ICS with those who used a high-dose ICS monotherapy.

Confounding variables

The following variables were identified in the literature as risk factors for congenital malformations and were considered potential confounders in our analysis: maternal age at the beginning of pregnancy (18–34 and <18 or ≥35 years),(29) receipt of social assistance during pregnancy (yes/no),(30) area of residence at delivery (rural/urban),(31, 32) chronic hypertension (yes/no),(33) diabetes mellitus (yes/no),(22, 33) exacerbation of asthma (defined as a filled prescription for OCS, an emergency department visit, or a hospitalization for asthma) three months before pregnancy (yes/no),(8) and short-acting beta$_2$-agonists (SABA) dose per week (0–3 or > 3 doses/week; one dose is equal to 200 μg of salbutamol) in the three months preceding pregnancy.(14)

Statistical analysis

The descriptive statistics for the characteristics of the pregnancies were calculated and compared between the combination therapy group and the ICS monotherapy group within each subcohort. We calculated the crude prevalence of any major or a specific major malformation within each subcohort.

The risk of major congenital malformations was compared between the combination therapy and the ICS monotherapy (reference) groups separately within the two subcohorts. We used generalized estimating equation (GEE) models with a logistic link and an exchangeable correlation matrix to estimate the crude and adjusted odds ratios (ORs) for major congenital malformations, with adjustment for all the potential confounders listed above and using the pregnancy as the unit of analysis. A GEE model was used to take into account the correlation between the consecutive pregnancies of individual women.(34) Of note, the risk measured in the analysis is an actual measure of major malformation prevalence since the data on the aborted fetuses with malformation is not recorded in the databases.

We conducted a sensitivity analysis that combined the two subcohorts together while adjusting for a variable indicating from which subcohort the pregnancy came. This sensitivity analysis was conducted to increase the power of the analysis compared with our primary stratified analysis. The adjusted results represent an overall comparison of the

combination therapy and the higher-dose ICS monotherapy. Finally, we used *post hoc* power calculations to identify the ORs that the study could detect with a power of 80%. These calculations were based on a test for the difference between two independent proportions with a type I error of 0.05, the number of pregnancies exposed to each of the contrasted treatment regimens in the subcohorts, and the percentage of pregnancies with a congenital malformation observed in the reference group (ICS monotherapy), and were performed with the PASS interface of the NCSS software (2007).(35) All other statistical analyses were performed with the SAS software, version 9.3 (SAS Institute Inc., Cary, NC).

Ethics approval

This research project was approved by the Ethics Committee of the *Hôpital du Sacré-Cœur de Montréal*. Authorization was obtained from the *Commission d'accès à l'information du Québec* before the information from the RAMQ and MED-ECHO databases was accessed and linked.

RESULTS

We first identified 6632 pregnancies (from 4619 asthmatic women) from the *Quebec Asthma and Pregnancy Database* that fulfilled our inclusion criteria, in women who were exposed to ICS during the first trimester (see Figure 1 for the selection process of the subcohorts). After applying the exclusion criteria, we retained 6355 pregnancies. From these, 1302 pregnancies (in 1249 women) were finally included in the two subcohorts (948 pregnancies in the moderate asthma subcohort and 354 in the severe asthma subcohort). Overall, 96 newborns (7.4%) with major malformations were detected during the first year of life among the 1302 pregnancies included in the two subcohorts.

The characteristics of the pregnancies included in the moderate and severe asthma subcohorts are presented in Table I. In both subcohorts, most of the women were 18–34 years of age, lived in urban areas, and did not suffer from chronic hypertension or diabetes mellitus. However, women in the severe asthma subcohort were more likely to have

suffered exacerbated asthma (p value <0.001) and to have used > 3 doses of SABA per week (p value <0.001) in the three months preceding the pregnancy than the women in the moderate asthma subcohort. In both subcohorts, the women treated with the ICS monotherapy were more likely to receive social assistance (p value <0.001), and to use > 3 doses of SABA per week (p value <0.05) than those treated with the combination therapy. In the severe asthma subcohort, we also observed that women exposed to the high-dose ICS monotherapy were older than women exposed to a combination therapy (p value <0.05).

In the moderate asthma subcohort, 21 cases (6.9%) of major congenital malformations were detected among the women treated with the combination therapy, whereas 46 cases (7.2%) were detected among women treated with the medium-dose ICS monotherapy. In the severe asthma subcohort, 14 cases (7.1%) of major congenital malformations were detected among women treated with the combination therapy, whereas 15 cases (9.6%) were detected among women treated with the high-dose ICS monotherapy. We present the distribution of specific groups of major congenital malformations in each subcohort in Table E2. In both subcohorts, cardiac malformations were the most frequent malformations, with prevalences varying between 2.0% for women treated with LABA plus medium-dose ICS and 3.2% for women treated with the high-dose ICS monotherapy.

In Table II, we present the crude and adjusted ORs for the moderate asthma subcohort. We observed no significant difference in the risk of major congenital malformations between women treated with the combination therapy and those treated with the medium-dose ICS monotherapy (adjusted OR: 1.1; 95% CI: 0.6–1.9). The receipt of social assistance during pregnancy was associated with an increased risk of major malformations (adjusted OR: 2.0; 95% CI: 1.2–3.5). None of the other variables included in the model were significantly associated with the risk of major malformations. With the sample size of this subcohort, we had a power of 80% to detect an OR of 1.9.

In Table III, we present the results for the severe asthma subcohort. Women treated with medium-dose ICS plus LABA had a nonsignificant 20% higher risk of major malformations than women treated with high-dose ICS (adjusted OR: 1.2; 95% CI: 0.5–2.7). This model also showed that women younger than 18 years or older than 34 years

were two times more likely to have a baby with a congenital malformation than women aged 18–34 years. With the sample size of this subcohort, we had a power of 80% to detect an OR of 2.4.

We present the results of the sensitivity analysis, in which we combined the two subcohorts, in Table IV. The use of the LABA plus ICS combination therapy did not entail a significantly increased risk of major malformations compared with the use of a higher-dose ICS monotherapy during the first trimester (adjusted OR: 1.0; 95% CI: 0.6–1.7). This model also revealed that the receipt of social assistance during pregnancy was associated with a significantly increased risk of major malformations (adjusted OR: 2.1; 95% CI: 1.3–3.4). With the sample size of the combined cohort, we had a power of 80% to detect an OR of 1.7.

DISCUSSION

This population-based comparative safety study showed that the risk of major malformations did not differ when a combination therapy of LABA plus ICS or a higher dose of ICS monotherapy was used in the first trimester of pregnancy. This result was consistent in both subcohorts of moderately and severely asthmatic pregnant women. Moreover, both the subcohort analysis and the combined secondary analysis showed similar results.

To the best of our knowledge, this study is the first to compare the risk of congenital malformations for different comparable treatment options for the management of moderate to severe asthma during pregnancy. Previous studies have compared women treated with LABA or ICS with either asthmatic women not exposed to the medication or nonasthmatic women.(13, 36) ICSs are the most frequently recommended controller therapy for the management of persistent asthma during pregnancy and their safety in terms of congenital malformations has been reported in 17 published studies.(36, 37) A recent meta-analysis reported no increased risk of congenital malformations with ICS use.(1) However, it included only three studies, “exposure” was defined as the use of an ICS at any dose versus no use anytime during pregnancy, and congenital malformations

also included minor ones. Nonetheless, in a previous study conducted by our group that was based on an earlier cohort (1990–2002) of asthmatic pregnant women, we found a 63% increased risk of congenital malformations (most prominently musculoskeletal and cardiac malformations) in women using high doses of ICS (> 500 μg/day fluticasone equivalents) compared with women who used low-to-moderate doses of ICS (> 0 to 500 μg/day) during the first trimester.(12)

Of eight published studies that examined maternal LABA use and congenital malformations, only one reported a significant association.(13) In a database-driven study published by our group that was based on an earlier cohort (1990–2002) of asthmatic pregnant women, we found that women exposed to LABA during the first trimester were at greater risk of giving birth to a baby with a major cardiac or major "other and unspecified malformation" (adjusted OR: 2.4; 95% CI: 1.1–5.1 or adjusted OR: 4.0; 95% CI: 1.3–12.2, respectively).(14) That study adjusted for asthma severity/control, but we could not rule out the presence of residual confounding by indication. The sample size of that earlier cohort (1990–2002) was not sufficient to compare the LABA plus ICS combination therapy with the higher-dose ICS monotherapy as we did in the present study. A common characteristic of all studies that have examined LABA use is their small sample sizes, and this is probably the result of the relatively recent introduction of these drugs into the markets and the controversies that have surrounded them.(38, 39) Importantly, caution should be used in interpreting the negative results of studies with small sample sizes.

The biological mechanisms of teratogenicity of ICS and LABA are still uncertain, but several hypotheses exist. A proportion of the ICS that enter the systemic circulation may cross the placenta and affects the fetus, also diffusion of fluorinated corticosteroids (e.g. fluticasone and budesonide) is even more rapid than other corticosteroids.(40-42) Since fetal endogenous levels of corticosteroids are much lower than maternal levels, even minimal diffusions to the fetus could have a considerable impact.(43) Evidence shows that corticosteroids influences maternal hypothalamic-pituitary-adrenal (HPA) activity, which may play a role in endocrine and metabolic alterations in the offspring.(44) The early presence of the glucocorticoid receptor in the fetus implies that corticosteroids may affect the fetus by the glucocorticoid receptor and lead to persistent disorders in endocrine and

metabolic control.(45) Animal models displayed potent teratogenicity of corticosteroids at doses less than or similar to those used in humans, with cleft palate being the primary malformation induced in most species.(42, 46) Corticosteroids are essential for normal differentiation and growth of epithelial cells, but supraphysiologic doses interrupt this process.(47). Regarding LABA, a probable teratogenic effect could arise from their potential effect on the corticosteroid function. It was demonstrated that LABA could induce the gene transcription effect of corticosteroids, which subsequently might increase their teratogenic effects.(48, 49)

Our primary objective was to compare the risk of major congenital malformations after two currently used—and probably widely used today—treatment options for the management of persistent moderate-to-severe asthma during pregnancy. We minimized any confounding by indication by performing the primary analysis within subcohorts of women with similar levels of asthma severity and by adjusting for baseline severity markers, but because there was no randomization of the treatment, we cannot be sure that there was no residual confounding. Because our objective was to examine the safety of two similar treatment regimens, we also cannot exclude the possibility that the use of either high-dose ICS or LABA is associated with a higher risk of major malformations than no use of these medications. However, such comparisons (use versus no use) are less clinically relevant because not treating a woman who requires high-dose ICS or the addition of LABA to a lower-dose ICS to control her asthma during pregnancy is clearly not a treatment option.

The present study has some important strengths. The use of two large administrative databases allowed us to access a large number of pregnancies in asthmatic women, from which we could establish our subcohorts and measure several potentially important confounders. Data on filled prescriptions, which were used to assess the women's exposure to asthma medications during pregnancy, were prospectively collected independently of the outcome, avoiding any recall bias, which is common in reproductive research. As mentioned earlier, we minimized confounding by indication by comparing two treatment regimens that have similar indications. However, the results of this study should be interpreted with consideration of the following limitations. The use of

medications was measured by medication claims, which might not reflect their actual intake. Moreover, we considered maternal LABA exposure as dichotomous (i.e., exposed or not exposed during the first trimester) because in practice, the dose prescribed varies little between patients. This definition might have diluted the exposure because not all women will adhere fully (100%) to their LABA prescription and this could have contributed to an underestimation of the impact of the ICS/LABA combination on the risk of major malformations. There is also a possibility of residual confounding arising from unmeasured risk factors for congenital malformations, such as smoking status, maternal obesity, over-the-counter medications, and some other environmental teratogens.(22) Another source of residual confounding is the absence of information on the provider classification of asthma severity, since this variables is not recorded in the databases. We had a statistical power of 80% to detect an OR of 1.9 in the moderate asthma subcohort and an OR of 2.4 in the severe asthma subcohort, and associations smaller than that might not have been detected in our primary analysis. However, the secondary analysis, which combined the two subcohorts, had power of 80% to detect an OR of 1.7. Finally, the cohort underrepresents women of higher socioeconomic status, which may limit the generalizability of the study results.

In summary, the risk of major congenital malformations was not higher among asthmatic pregnant women treated with a LABA plus ICS combination therapy than among women treated with an ICS monotherapy at a higher dose during the first trimester. These reassuring results are consistent with asthma management guidelines, and provide scientific evidence to help physicians and mothers make evidence-based treatment decisions during pregnancy. These results should encourage women to continue to take their asthma medications when required to control their asthma during pregnancy, and as suggested by previous research evidence, this will increase the likelihood of healthy pregnancies and newborns.

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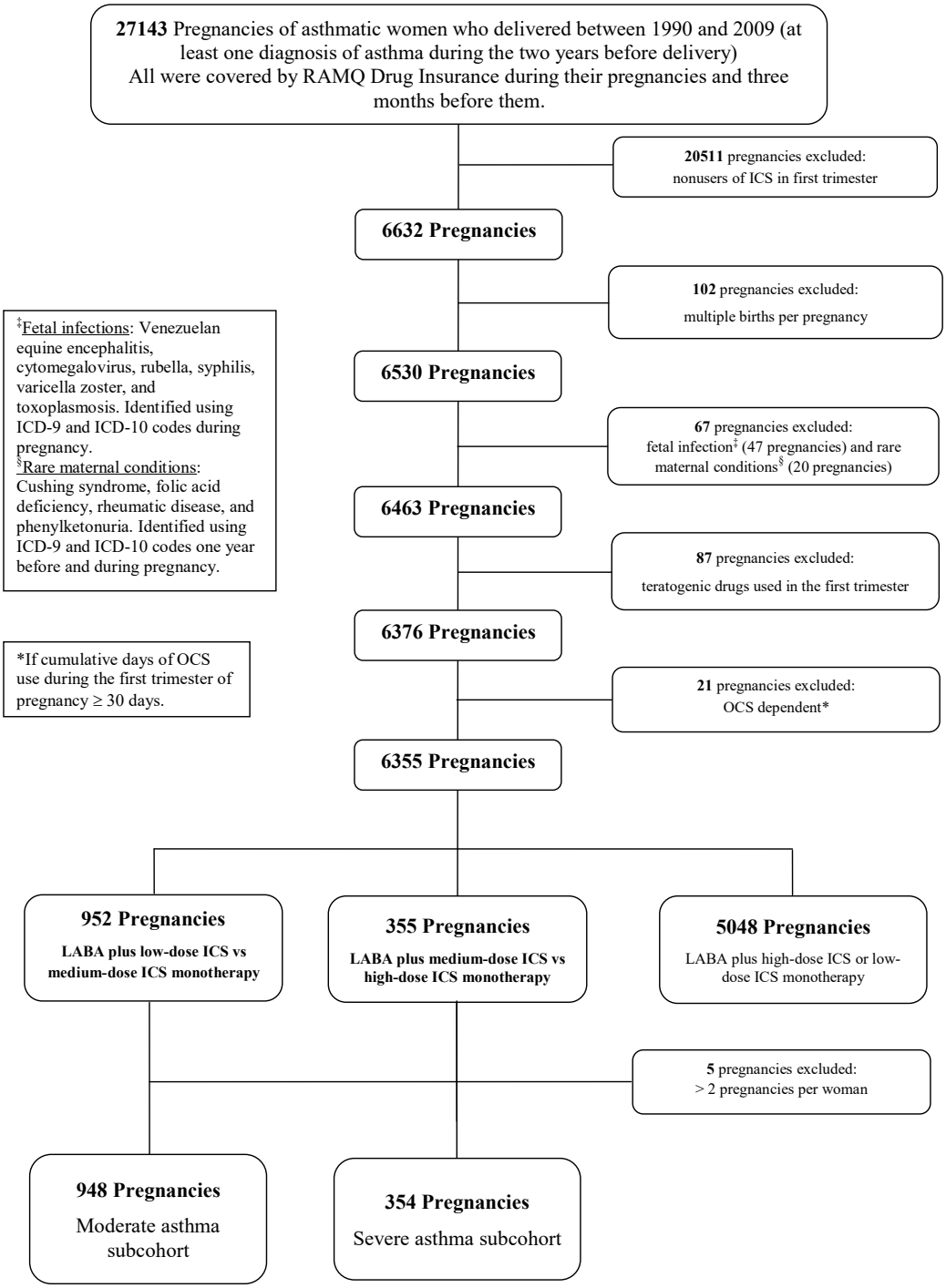

Figure 1. Cohort flow diagram and the subcohort selection process.

Table I. Distribution of maternal characteristics according to exposure to ICS and LABA during the first trimester of pregnancy in each subcohort of asthmatic women

	Moderate asthma subcohort			Severe asthma subcohort		
	LABA plus low-dose ICS	Medium-dose ICS	*P* value	LABA plus medium-dose ICS	High-dose ICS	*P* value
Total number of pregnancies in the subcohort (%)	305 (32.2)	643 (67.8)		198 (55.9)	156 (44.1)	
	Number of pregnancies (%)					
Maternal age (years) at the beginning of pregnancy						
18–34	268 (87.9)	544 (84.6)	0.180	171 (86.4)	119 (76.3)	0.014
< 18 or ≥ 35	37 (12.1)	99 (15.4)		27 (13.6)	37 (23.7)	
Receipt of social assistance during pregnancy	130 (42.6)	374 (58.2)	< 0.001	89 (45.0)	116 (74.4)	< 0.001
Urban area of residence at delivery	243 (79.7)	497 (77.3)	0.409	152 (76.8)	125 (80.1)	0.447
Chronic hypertension	7 (2.3)	16 (2.5)	0.857	8 (4.0)	6 (3.9)	0.926
Diabetes mellitus	12 (3.9)	33 (5.1)	0.418	8 (4.0)	12 (7.7)	0.139
Exacerbation of asthma three months before pregnancy	46 (15.1)	75 (11.7)	0.141	35 (17.7)	33 (21.2)	0.410
SABA doses/week three months before pregnancy						
0–3	144 (47.2)	249 (38.7)	0.013	59 (29.8)	21 (13.5)	< 0.001
> 3	161 (52.8)	394 (61.3)		139 (70.2)	135 (86.5)	

LABA: long-acting beta$_2$-agonists, ICS: inhaled corticosteroids

Table II. Crude and adjusted odds ratios for major congenital malformations in the moderate asthma subcohort

	No. preg.	*No. cases (%)*	*Crude OR (95% CI)*	*Adjusted OR (95% CI)*
LABA plus low-dose ICS	305	21 (6.9)	**0.9 (0.5–1.6)**	**1.1 (0.6–1.9)**
Medium-dose ICS	643	46 (7.2)	Reference	Reference
Maternal age (years) at the beginning of pregnancy				
18–34	812	56 (6.9)	Reference	Reference
< 18 or ≥ 35	136	11 (8.1)	1.2 (0.6–2.4)	1.1 (0.6–2.2)
Receipt of social assistance during pregnancy				
Yes	504	46 (9.1)	2.0 (1.2–3.4)	2.0 (1.2–3.5)
No	444	21 (4.7)	Reference	Reference
Area of residence at delivery				
Urban	740	47 (6.4)	Reference	Reference
Rural	208	20 (9.6)	1.5 (0.9–2.7)	1.7 (0.9–2.9)
Chronic hypertension				
Yes	23	3 (13.0)	2.0 (0.6–7.0)	1.9 (0.6–6.1)
No	925	64 (6.9)	Reference	Reference
Diabetes mellitus				
Yes	45	5 (11.1)	1.6 (0.5–4.7)	1.4 (0.5–4.2)
No	903	62 (6.9)	Reference	Reference
Exacerbation of asthma three months before pregnancy				
Yes	121	9 (7.4)	1.1 (0.5–2.3)	1.0 (0.5–2.0)
No	827	58 (7.0)	Reference	Reference
SABA doses/week three months before pregnancy				
0–3	393	26 (6.6)	Reference	Reference
> 3	555	41 (7.4)	1.1 (0.7–1.9)	1.0 (0.6–1.8)

LABA: long-acting beta$_2$-agonists, ICS: inhaled corticosteroids, preg: pregnancies

Table III. Crude and adjusted odds ratios for major congenital malformations in the severe asthma subcohort

	No. preg.	*No. cases (%)*	*Crude OR (95% CI)*	*Adjusted OR (95% CI)*
LABA plus medium-dose ICS	198	14 (7.1)	**0.7 (0.3–1.5)**	**1.2 (0.5–2.7)**
High-dose ICS	156	15 (9.6)	Reference	Reference
Maternal age (years) at the beginning of pregnancy				
18–34	290	20 (6.9)	Reference	Reference
< 18 or ≥ 35	64	9 (14.1)	2.2 (1.0–5.1)	2.4 (1.0–5.5)
Receipt of social assistance during pregnancy				
Yes	205	22 (10.7)	2.4 (1.0–5.9)	2.5 (0.9–6.9)
No	149	7 (4.7)	Reference	Reference
Area of residence at delivery				
Urban	277	21 (7.6)	Reference	Reference
Rural	77	8 (10.4)	1.4 (0.6–3.3)	1.6 (0.6–4.1)
Chronic hypertension				
Yes	14	2 (14.3)	1.9 (0.4–9.1)	1.6 (0.4–7.5)
No	340	27 (7.9)	Reference	Reference
Diabetes mellitus				
Yes	20	0 (0.0)	Not included	Not included
No	334	29 (8.7)	Reference	Reference
Exacerbation of asthma three months before pregnancy				
Yes	68	5 (7.4)	0.9 (0.3–2.4)	0.8 (0.3–2.3)
No	286	24 (8.4)	Reference	Reference
SABA doses/week three months before pregnancy				
0–3	80	2 (2.5)	Reference	Reference
> 3	274	27 (9.9)	4.3 (1.0–18.3)	3.9 (0.9–15.9)

LABA: long-acting beta$_2$-agonists, ICS: inhaled corticosteroids, preg: pregnancies

Table IV. Crude and adjusted odds ratios for major congenital malformations in the combined cohort

	No. preg.	*No. cases (%)*	*Crude OR (95% CI)*	*Adjusted OR (95% CI)*
LABA plus ICS combination therapy	503	35 (7.0)	**0.9 (0.6–1.4)**	**1.0 (0.6–1.7)**
Higher-dose ICS monotherapy	799	61 (7.6)	Reference	Reference
ICS dose group				
Moderate asthma	948	67 (7.1)	Reference	Reference
Severe asthma	354	29 (8.2)	1.2 (0.8–1.9)	1.1 (0.7–1.8)
Maternal age (years) at the beginning of pregnancy				
18–34	1102	76 (6.9)	Reference	Reference
< 18 or ≥ 35	200	20 (10.0)	1.6 (0.9–2.6)	1.5 (0.9–2.5)
Receipt of social assistance during pregnancy				
Yes	709	68 (9.6)	2.1 (1.3–3.3)	2.1 (1.3–3.4)
No	593	28 (4.7)	Reference	Reference
Area of residence at delivery				
Urban	1017	68 (6.7)	Reference	Reference
Rural	285	28 (9.8)	1.5 (0.9–2.3)	1.6 (1.0–2.5)
Chronic hypertension				
Yes	37	5 (13.5)	2.1 (0.8–5.4)	1.8 (0.7–4.4)
No	1265	91 (7.2)	Reference	Reference
Diabetes mellitus				
Yes	65	5 (7.7)	1.0 (0.4–2.6)	0.8 (0.3–2.3)
No	1237	91 (7.4)	Reference	Reference
Exacerbation of asthma three months before pregnancy				
Yes	189	14 (7.4)	1.1 (0.6–2.0)	1.0 (0.5–1.8)
No	1113	82 (7.4)	Reference	Reference
SABA doses/week three months before pregnancy				
0–3	473	28 (5.9)	Reference	Reference
> 3	829	68 (8.2)	1.4 (0.9–2.2)	1.3 (0.8–2.0)

LABA: long-acting beta$_2$-agonists, ICS: inhaled corticosteroids, preg: pregnancies

Table E1. Specific groups of major malformations and their related ICD-9/ICD-10 codes

	ICD-9 code	ICD-10 code
Nervous system	740, 741, 742	Q00, Q01, Q02, Q03, Q04, Q05, Q06, Q07
Cardiac	745, 746	Q20, Q21, Q22, Q23, Q24
Circulatory system	747	Q25, Q26, Q27, Q28
Respiratory system	748	Q30, Q31, Q32, Q33, Q34
Eye	743	Q10, Q11, Q12, Q13, Q14, Q15,
Ear, face, and neck	744	Q16, Q17, Q18
Cleft palate and cleft lip	749	Q35, Q36, Q37
Digestive system	750, 751	Q38, Q39, Q40, Q41, Q42, Q43, Q44, Q45
Genital organs	752	Q50, Q51, Q52, Q53, Q54, Q55, Q56, Q640
Urinary system	753	Q60, Q61, Q62, Q63, Q64[b]
Limbs	754.4, 754.5, 754.6, 754.7, 755	Q658, Q659, Q66, Q682, Q683, Q684, Q685, Q69, Q70, Q71, Q72, Q73, Q74
Musculoskeletal	754a, 756	Q65[c], Q66, Q67, Q68[d], Q75, Q76, Q77, Q78, Q79
Integument	757	Q80, Q81, Q82, Q83, Q84
Chromosomal	758	Q90, Q91, Q92, Q93, Q94, Q95, Q96, Q97, Q98, Q99
Other congenital anomalies	759	Q85, Q86, Q87, Q89

A congenital malformation was defined as major if it was life-threatening or could cause major cosmetic defects. When a malformation could be classified as major or minor by the geneticist, we considered it major only if there was at least one hospitalization with a primary or an admission diagnosis related to this malformation in the MED-ECHO database during the first year of life of the newborn.

ICD: International Classification of Diseases.

[a] Including all '754' codes except those mentioned above for limb malformations.

[b] Including all 'Q64' codes except 'Q640'.

[c] Including all 'Q65' codes except those mentioned above for limb malformations.

[d] Including all 'Q68' codes except those mentioned above for limb malformations.

Table E2. Distribution of major congenital malformations in the moderate asthma and severe asthma subcohorts

	Moderate asthma subcohort				Severe asthma subcohort			
	LABA plus low-dose ICS		Medium-dose ICS		LABA plus medium-dose ICS		High-dose ICS	
	No.	***%***	***No.***	***%***	***No.***	***%***	***No.***	***%***
At least one major malformation[a]	**21**	**6.9**	**46**	**7.2**	**14**	**7.1**	**15**	**9.6**
Specific major malformations								
Nervous system	4	1.3	4	0.6	2	1.0	2	1.3
Cardiac	8	2.6	15	2.3	4	2.0	5	3.2
Circulatory system	1	0.3	2	0.3	2	1.0	2	1.3
Respiratory system	3	1.0	4	0.6	1	0.5	0	0.0
Eye	0	0.0	1	0.2	0	0.0	0	0.0
Ear, face, and neck	0	0.0	0	0.0	0	0.0	0	0.0
Cleft palate and cleft lip	1	0.3	3	0.5	0	0.0	0	0.0
Digestive system	0	0.0	11	1.7	1	0.5	2	1.3
Genital organs	1	0.3	2	0.3	1	0.5	1	0.6
Urinary system	0	0.0	3	0.5	3	1.5	6	3.9
Limbs	0	0.0	1	0.2	0	0.0	2	1.3
Musculoskeletal[b]	2	0.7	6	0.9	1	0.5	3	1.9
Integument	4	1.3	2	0.3	2	1.0	2	1.3
Chromosomal	1	0.3	3	0.5	2	1.0	1	0.6
Other congenital anomalies	2	0.7	2	0.3	0	0.0	2	1.3

LABA: long-acting beta$_2$-agonists, ICS: inhaled corticosteroids

[a] The total sum of all major specific malformations exceeded the number of cases (96 infants with major malformations) because an infant could have more than one malformation.

[b] Including all musculoskeletal malformations except limb malformations.

5.3. Article on the case ascertainment definitions of major malformations

5.3.1 Published Manuscript

The Impact of Different Case Ascertainment Definitions on the Prevalence of Major Congenital Malformations and their Association with Asthma during Pregnancy

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The Impact of Different Case Ascertainment Definitions on the Prevalence of Major Congenital Malformations and their Association with Asthma during Pregnancy

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Running Head: Case Ascertainments of Major Malformations

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ABSTRACT

Purpose: To compare the prevalence of major malformations using different case ascertainment definitions and to evaluate their impact on maternal asthma-major malformations association. **Methods**: A cohort of pregnancies with and without asthma between 1990 and 2010 was formed. We used two classification methods: the Two step Congenital Malformation Classification (TCMC) and the Canadian Congenital Anomalies Surveillance System (CCASS). Within each method, three case definitions were compared: (1) ≥1 diagnosis in the hospital database; (2) ≥1 diagnosis in the hospital database or ≥2 in the medical claims; and (3) ≥1 diagnosis in the hospital database or ≥1 in the medical claims. We calculated the prevalence of major malformations and adjusted odds ratios (aORs) for maternal asthma association. **Results:** Of 467,946 pregnancies, 12.3% were with active asthma. The prevalence estimates were: TCMC 5.10%–7.08% and CCASS 7.03%–10.57%. Asthma-major malformations association was weaker with the CCASS (aOR 1.14–1.20) versus TCMC (aOR 1.22–1.26). **Conclusions**: The case ascertainment definitions with ≥ 1 hospitalization are likely to be the most reliable in similar administrative databases. The case ascertainment definition had a considerable impact on the prevalence of major malformations, but hardly influenced the aORs. Future studies should formally assess the validity of the case ascertainment definitions and allow generalizability to other maternal exposures.

Keywords: Congenital malformations, case definitions, asthma, pregnancy, administrative databases.

Significance

What is already known on this subject?

Several case ascertainment definitions of major congenital malformations are currently being used in perinatal epidemiology, but few studies compared their impacts.

What this study adds?

In this study, six different case ascertainment definitions were compared, resulting in a considerable impact on prevalence estimates, but little on the measures of association. The prevalence estimates ranged between 5.1% and 10.6%, with medical claims playing a major role in the prevalence increase. The case ascertainment definitions using ≥ 1 diagnosis of major malformation recorded in hospital databases or discharge summary sheets are the most recommended since they have the least chance of including misclassified cases yet providing adequate results.

Ethical Statement

This research project was approved by the Ethics Committee of the *Hôpital du Sacré-Cœur de Montréal*. Authorization was obtained from the *Commission d'Accès à l'Information du Québec* to access and link the RAMQ and MED-ECHO databases.

INTRODUCTION

Computerized administrative databases have become an important source of data in perinatal epidemiology, with increasing number of studies evaluating the impact of pregnancy exposures on perinatal outcomes including congenital malformations.(Correa & Kirby, 2010) Major malformations are considered among the leading causes of infant, fetal, and post neonatal mortality in North America and Europe. (Khoshnood et al., 2011; *Public Health Agency of Canada. Perinatal Health Indicators for Canada 2013: a Report of the Canadian Perinatal Surveillance System. Ottawa, 2013.*) However, discrepancies are present in the estimated prevalence of major malformations in prior reports. These differences are attributed to several factors, including the source of data, the diagnostic codes validity, the classification method, and the period of assessment (e.g. at birth or during the 1st year of life).(Bedard, Lowry, Sibbald, et al., 2012; Hobbs, Hopkins, & Simmons, 2001; Rasmussen & Moore, 2001) All these factors should be considered in specifying and developing the case ascertainment definitions in epidemiological studies. Typically, a case ascertainment definition for congenital malformations is pre-specified by the investigators and operated through applying an algorithm to the raw data in the databases (e.g. medical records with congenital malformations diagnoses) in order to capture the required cases.

Quebec is the second largest province in Canada and several health administrative databases were established and increasingly being used in epidemiological research. Among others, the *Régie de l'Assurance-Maladie du Québec* (RAMQ) Medical Claims database records data on medical services paid on a fee-for-service basis for all residents of Quebec and the *Maintenance et Exploitation des Données pour l'Étude de la Clientèle Hospitalière* (MED-ECHO) database records data related to all acute care hospitalisations in the province. Both databases have been used previously for congenital malformations research.(Berard et al., 2007; De Wals, Rusen, Lee, Morin, & Niyonsenga, 2003; Eltonsy, Forget, Beauchesne, & Blais, 2014; Tairou, De Wals, & Bastide, 2006) The accuracy of congenital malformations diagnoses recorded in MED-ECHO and RAMQ databases was reported in two studies.(Blais, Berard, Kettani, & Forget, 2013; Kulaga & Berard, 2010)

Kulaga et al. reported a 60% relative agreement between MED-ECHO, RAMQ, Births and Deaths Registry records, and the mothers' reports.(Kulaga & Berard, 2010) Comparing the malformations diagnoses recorded in the linked MED-ECHO and RAMQ databases with the infants' medical charts (the gold standard), Blais et al. reported a positive predictive value (PPV) of 78.1% for major malformations among asthmatic women and 69.0% among non-asthmatic women.(Blais et al., 2013) However, the aforementioned studies did not investigate the accuracy of the diagnoses reported in each database separately. It is currently unknown whether one of these two databases has a higher accuracy of malformations diagnoses over the other. In Quebec, medical billing claims database is used mainly for billing and administrative purposes with the diagnoses information being recorded by physicians and not mandatory. While in the hospitalizations database, the diagnoses recording is mandatory and performed by trained medical archivists.

Beside the variation in the prevalence estimates, the case ascertainment definitions might also influence the estimates for the associations between maternal exposures and congenital malformations. The two most likely common scenarios of deviations from the truth have different causes and effects. Incomplete and non-differential ascertainment of congenital malformations will hardly affect the effect estimates themselves but will affect the precision. On the other hand, over-ascertainment by inclusion of non-malformed newborns or newborns with minor malformations (i.e. false positives) could lead to underestimating of the true impact of the exposure and lower observed effect estimates. Therefore, the objective of the current study was to compare the prevalence of major malformations using different case ascertainment definitions that vary by the source of data and the classification method. We also evaluated the impact of these definitions on the association between maternal asthma and major malformations.

METHODS

Data sources

We used the Quebec Asthma and Pregnancy Database, including all pregnancies in all women with ≥ 1 asthma diagnosis (International Classification of Diseases [ICD] 9th

Revision [ICD-9] code 493 or ICD-10 code J45) in the 2-year period preceding one of their deliveries and all pregnancies of a 4-times-larger random sample of other women who delivered between January 1, 1990 and March 31, 2010 in Quebec (see Figure 1). The database includes 583,071 pregnancies, representing about 35% of all births in the province in this period of time.(*Statistics Canada-Components of population growth, Canada, provinces and territories. http://www5.statcan.gc.ca/cansim/a26?lang=eng&id=510004*) External validity was not assessed per se, but including all pregnancies from asthmatic women and a large random sample from non-asthmatic women should provide high external validity.

This database contains data extracted from the MED-ECHO and RAMQ databases. Since the RAMQ Medical Claims database records data on all medical visits, including diagnosis codes and procedures, for all residents of Quebec, we hypothesized that it contained valuable information on congenital malformations that could be missing from the MED-ECHO database. The diagnoses recorded in the MED-ECHO database are routinely collected by trained medical archivists using the ICD-10 revised for Canada (ICD-10-CA) since 2006, and using the ICD-9 before 2006.The RAMQ Medical Claims database records diagnoses coded with ICD-9 codes. We obtained data from the RAMQ and MED-ECHO databases from January 1, 1988 to March 30, 2010 for the mothers, and from birth to March 30, 2010 for the offspring.

Study cohort and asthma definition

A cohort of pregnancies in women with active asthma and women without asthma was selected. Asthma was defined as ≥ 1 asthma diagnosis (ICD-9 code 493, except 493.2, or ICD-10 code J45) recorded during a hospitalization, or ≥ 2 medical claims associated with an asthma diagnosis within 2 consecutive years between 1988 and the delivery.(Gershon et al., 2009) This operational definition of asthma was previously validated and showed a sensitivity of 83.8% and a specificity of 76.5%.(Gershon et al., 2009) Asthma was considered to be present during pregnancy (i.e. active asthma) if there was at least one asthma diagnosis within 2 years before delivery.The pregnancy was considered as non-asthmatic if the woman had no diagnosis of asthma recorded in either database between 1988 and the delivery. The pregnancies inclusion criteria were: (1) a pregnancy with

delivery between January 1, 1990 and March 31, 2009 (allowing 1 year of data available after birth to assess congenital malformations); (2) maternal age at the beginning of pregnancy of 15–45 years; (3) gestational duration of 20–45 weeks; and (4) fulfillment of the definitions for the presence/absence of active asthma during pregnancy. Quadruplet births from a single pregnancy were excluded to avoid zero cells and pregnancies missing the mother–infant link were excluded.

Classification of congenital malformations

Congenital malformations were identified using the ICD-9 and ICD-10 codes for congenital malformations recorded in MED-ECHO and RAMQ databases at birth and during the 1st year of life for live births (≥ 20 weeks gestation) and at birth for stillbirths. We compared two methods for the classification of congenital malformations. The first, the Two-step Congenital Malformation Classification (TCMC) method, which was developed specifically for research and used in previous perinatal epidemiological studies.(Blais, Beauchesne, Lemiere, & Elftouh, 2009; Blais, Kettani, Elftouh, & Forget, 2010; Eltonsy et al., 2014) Briefly, to facilitate the development of a complete and comprehensive list of congenital malformations, a system specific malformations list was primarily compared with a list provided by the Collaborative Perinatal Group.(Heinonen, Slone, & Shapiro, 1977) The full list was then verified by a geneticist from the Centre Hospitalier Universitaire Sainte-Justine for exactness and completeness. , The first step included classifying congenital malformations as major, minor, or major/minor. A malformation was defined as "major" if it was life-threatening or could cause major cosmetic defects. As judged by the geneticist, the "major/minor" category includes malformations that vary in their severity, depending on the condition of each case. The "minor" category includes malformations not classified in the major or major/minor categories. In the second step, the major/minor malformations were reclassified as major if the malformation was associated with at least one hospitalization with a primary or an admission diagnosis in the MED-ECHO database in the first year of life of the baby or as minor if not.

The second method was the national Canadian Congenital Anomalies Surveillance System (CCASS) method, which has been described in more detail in the CCASS periodic

reports.(*Public Health Agency of Canada. Congenital Anomalies in Canada 2013 : A Perinatal Health Surveillance Report. Ottawa, 2013.*) Briefly, the ICD-9 and ICD-10 codes for congenital malformations were classified into 14 categories of system-specific malformations. Then, preselected diagnostic codes for minor malformations were excluded from these categories. Notably, the CCASS method is the Canadian national method of ascertainment which is currently used with several provincial databases. The CCASS uses discharge abstract data (DAD) on newborns, collected from provincial and territorial hospitals via the Canadian Institute for Health Information (CIHI) and Québec's MED-ÉCHO. The CCASS was originally developed for surveillance purposes. Online Resource 1 provides a complete description and comparison of the two classification methods. Congenital malformations were categorized into 16 system-specific categories, as presented in Online Resource 1. As presented in Table e1, the TCMC and the CCASS methods perfectly agree in the classification codes for nervous system, cardiac system, orofacial clefts, and Down syndrome. The rest of the system-specific malformations have codes that vary by the classification method.

Case ascertainment definitions of congenital malformations

We sought to compare case ascertainment definitions that differ by the source of data (i.e. diagnoses recorded in a hospital database [MED-ECHO] or in a medical billing claims database [RAMQ]) and the classification method (i.e. the TCMC or CCASS methods). We developed 3 case ascertainment definitions for each classification method, and they varied in the inclusion criteria from the strictest (i.e. using only hospital database) to the least strict (i.e. using ≥ 1 diagnosis from either the medical claims database or the hospital database). Using this methodology, we aimed at evaluating the separate effect of changing the classification method and changing the source of data.

We used six case ascertainment definitions: (1) TCMC: ≥ 1 major malformation diagnosis recorded in the hospital database; (2) TCMC: ≥ 2 major malformation diagnoses recorded in the medical claims database or ≥ 1 major malformation diagnosis recorded in the hospital database; (3) TCMC: ≥ 1 major malformation diagnosis recorded in the medical claims database or ≥ 1 major malformation diagnosis recorded in the hospital database; (4) CCASS: ≥ 1 major malformation diagnosis recorded in the hospital database; (5) CCASS:

≥ 2 major malformation diagnoses recorded in the medical claims database or ≥ 1 major malformation diagnosis recorded in the hospital database; and (6) CCASS: ≥ 1 major malformation diagnosis recorded in the medical claims database or ≥ 1 major malformation diagnosis recorded in the hospital database. The six case ascertainment definitions are summarized in Table 1.

Statistical analysis

The characteristics of the pregnancies were compared using descriptive statistics. We calculated the prevalence of major malformations using the six case ascertainment definitions. Next, we compared the prevalence of major malformations between pregnancies of women with active asthma and non-asthmatic women. We used generalized estimating equation (GEE) models with a logistic link and pregnancy as the unit of analysis to estimate crude and adjusted odds ratios (cOR, aOR) for major malformations with 95% confidence intervals (CI). GEE models were used to account for the correlation between consecutive pregnancies in individual women.(Zeger, Liang, & Albert, 1988) Adjusted models contained risk factors for congenital malformations, including maternal age at the start of pregnancy (18–34 and < 18 or > 35 years),(Gill et al., 2012) receipt of social assistance at the start of pregnancy (yes/no),(Yang, Carmichael, Canfield, Song, & Shaw, 2008) area of residence at delivery (rural/urban),(Langlois, Scheuerle, Horel, & Carozza, 2009; Messer et al., 2010) multiple pregnancy (yes/no), and the following maternal co-morbidities identified from diagnoses recorded in the MED-ECHO or RAMQ databases up to 1 year before pregnancy: chronic hypertension (yes/no),(Liu et al., 2013) diabetes mellitus (yes/no),(Brent, 2001; Liu et al., 2013) and epilepsy (yes/no).(Brent, 2001) All statistical analyses were performed with SAS software, version 9.3 (SAS Institute Inc., Cary, NC).

RESULTS

Of 467,946 pregnancies eligible for the study, 57,766 (12.3%) were in women with active asthma and 410,180 (87.7%) were in non-asthmatic women. The selection process is summarized in Figure 1. The characteristics of the pregnancies in women with active asthma and non-asthmatic women are presented in Table 2. Most of the women in both groups were 18–34 years old and lived in urban areas. However, women with active

asthma were more likely to receive social assistance and to suffer from other chronic diseases. Table 3 shows the prevalence of major malformations according to each case ascertainment definition. The prevalence of major malformations ranged from 5.1% to 7.1% using the TCMC method and from 7.0% to 10.6% using the CCASS method. The prevalence of major malformations increased when medical claims data were added to the hospitalization data. Additionally, the prevalence of major malformations was 37.8%, 42.4%, and 49.3% higher with the CCASS method than with the TCMC classification method for the case ascertainment definitions ≥ 1 major malformation diagnosis recorded in the hospital database; ≥ 2 major malformation diagnoses recorded in the medical claims database or ≥ 1 major malformation diagnosis recorded in the hospital database; and ≥ 1 major malformation diagnosis recorded in the medical claims database or ≥ 1 major malformation diagnosis recorded in the hospital database, respectively.

Table 3 also shows the prevalence of system-specific major malformations. The most prevalent categories were cardiac malformations, and limb and musculoskeletal malformations, regardless of the case ascertainment definition. Only four categories (neural tube defects, urinary system malformations, orofacial clefts, and Down syndrome) had prevalence that were relatively not affected by the different definitions. However, some categories were strongly affected by the data source (i.e. cardiac, central nervous system, eye, and other chromosomal malformations), other categories were affected by the classification method (i.e. limb and musculoskeletal, digestive system, integument, and ear, face and neck malformations), while others were affected by both (i.e. circulatory system, respiratory system, genital organ, and other and unspecified malformations).

Figure 2 shows the cORs and aORs for the association between maternal asthma and major malformations using all six case ascertainment definitions. The number and percentage of cases per group are presented in Online Resource 2. Using the TCMC method, maternal asthma was significantly associated with an increased prevalence of major malformations regardless of the definitions, with aORs ranging from 1.22 to 1.26. Using the CCASS method, maternal asthma was also significantly associated with an increased prevalence of major malformations regardless of the definitions, although the aORs were smaller and varied more, ranging from 1.14 to 1.20. Regardless of the classification method, adding

medical claims data to the hospitalization data had little impact on the effect size of the aORs.

DISCUSSION

In the current study, we compared six different case ascertainment definitions for major malformations that differ by the source of data and the classification method. Adding ≥ 1 or 2 medical claim diagnoses to hospital-based diagnoses increased the prevalence of major malformations by 10.0% and 38.8%, respectively, for the TCMC method and by 13.7% and 50.4%, respectively, for the CCASS method. The classification method itself influenced the prevalence with increases from 37.8% to 49.3% with the CCASS as compared with the TCMC method. We also observed weaker estimates of the association with maternal asthma with the CCASS (aORs 1.14–1.20) versus the TCMC (aORs 1.22–1.26) method, even though the prevalence was always greater with the CCASS method.

Using hospital, vital statistics, and medical genetic departments databases, Bedard et al. reported congenital heart defects prevalence of 5.59 per 1000 births, that increased to 12.42 per 1000 births when outpatient pediatric cardiology database and terminations of pregnancy data were added.(Bedard, Lowry, Sibbald, et al., 2012) Others have evaluated the accuracy of diagnoses of congenital malformations recorded in administrative databases.(Bedard, Lowry, & Sibbald, 2012; Cooper et al., 2008; Metcalfe, Sibbald, Lowry, Tough, & Bernier, 2014; Rasmussen & Moore, 2001; Salemi et al., 2011) Metcalfe et al., using a provincial database from Canada, reported an accurate identification rate of 86.9% for congenital malformations in the hospitalization database versus 51.1% in outpatient database.(Metcalfe et al., 2014) In other prior reports, an estimated 5%–20% of cases of major malformations were false positives.(Bedard, Lowry, Sibbald, et al., 2012; Callif-Daley, Huether, & Edmonds, 1995; Metcalfe et al., 2014; Salemi et al., 2011) Using linked data from Quebec, Kulaga et al. reported an agreement of 60% with maternal reports of major malformations.(Kulaga & Berard, 2010) Moreover, Blais et al. compared linked hospital and medical claims databases with data from the infants' medical charts, and reported a PPV of 78.1% for major malformations in asthmatic women and 69.0% in non-asthmatics.(Blais et al., 2013) Of note, the above-mentioned studies have investigated

the accuracy of the diagnoses per se, but did not compare the impact of different case ascertainment definitions on the prevalence and association estimates as in the current study.

We observed an increase of 10.0%–13.7% in the prevalence of major malformations for the case ascertainment definition of ≥ 1 hospitalization diagnosis or ≥ 2 medical claim diagnoses as compared to ≥ 1 hospitalization diagnosis. Meanwhile, the least strict definition of ≥ 1 hospitalization diagnosis or ≥ 1 medical claim diagnosis resulted in the greatest increase in the prevalence (by 38.8%–50.4%). It is possible that the medical claims included some suspected malformations that were never confirmed. For this reason, we believe that the definition based on ≥ 1 hospitalization diagnosis most probably includes the fewest false positives.

In terms of classification methods, the CCASS led to higher prevalence of major malformations than the TCMC, because some minor malformations were classified as major malformations. Indeed, the prevalence of some categories (e.g. limb and musculoskeletal, and ear, face and neck) were three times higher using the CCASS method. The number of misclassified cases is difficult to determine due to the lack of a gold standard, but we hypothesize that the specificity of the CCASS is lower than that of the TCMC method. This outcome misclassification increases the estimated prevalence and can lead to an information bias (non-differential misclassification) that would underestimate the impact of an exposure on the prevalence of major malformations. (Rothman, Greenland, & Lash, 2008) This phenomenon was indeed observed with the aORs being closer to the null with the CCASS than with the TCMC method. There is a well-documented association between asthma and major malformations, with an increased prevalence of about 20%–30% relative to pregnancies in non-asthmatic women.(Blais & Forget, 2008; Blais et al., 2010; Murphy et al., 2013) The results obtained using the TCMC method were more consistent with these results. For these reasons, we believe that the TCMC method includes the fewest false positives.

The present study has some important strengths. It comprised one of the largest administrative-linked pregnancy databases including more than 500,000 pregnancies over 20 years. The study was the first to examine the number of additional cases of major malformations identified in outpatient medical claims database in Quebec, and the first to

compare the CCASS classification method used for national surveillance to the TCMC method designed specifically for perinatal research. While we used Quebec databases, the results are generalizable and can straightforwardly reflect to different settings where similar health administrative databases are available.

The current study has some limitations. The lack of a gold standard means we were unable to estimate the PPV or the negative predictive value for the case ascertainment definitions. Although the accuracy of the diagnoses is expected to be greater in a hospital database (because of active and prospective data entry by trained medical archivists) than in a medical claims database maintained mainly for billing purposes, the recording of all diagnoses, including suspected and confirmed cases, could lead to false-positive cases. However, there is no reason to believe that the recording was differential between asthmatic and non-asthmatic women, reducing this potential bias towards the null. Finally, it is unlikely that one maternal exposure (i.e. asthma) will result in the increase of all major malformations categories. We used all major malformations combined only as an empirical example that provided the largest number of cases and the capacity to compare with previous studies. Future research should explore the associations between maternal exposures and specific categories of malformations (e.g. maternal diabetes and cardiac defects).

In conclusion, our study showed that the case ascertainment definitions had a considerable impact on the prevalence of major malformations, but a small influence on the aORs. The case ascertainment definition based on ≥ 1 hospitalization diagnosis combined with the TCMC is the preferred method, since it has the least chance of including misclassified and false positive cases. These results could assist in guiding future research on congenital malformations and the comparative effectiveness and safety of drug therapies during pregnancy. Future studies are needed to formally assess the validity of the proposed case ascertainment definitions and to estimate their impact on other maternal exposures.

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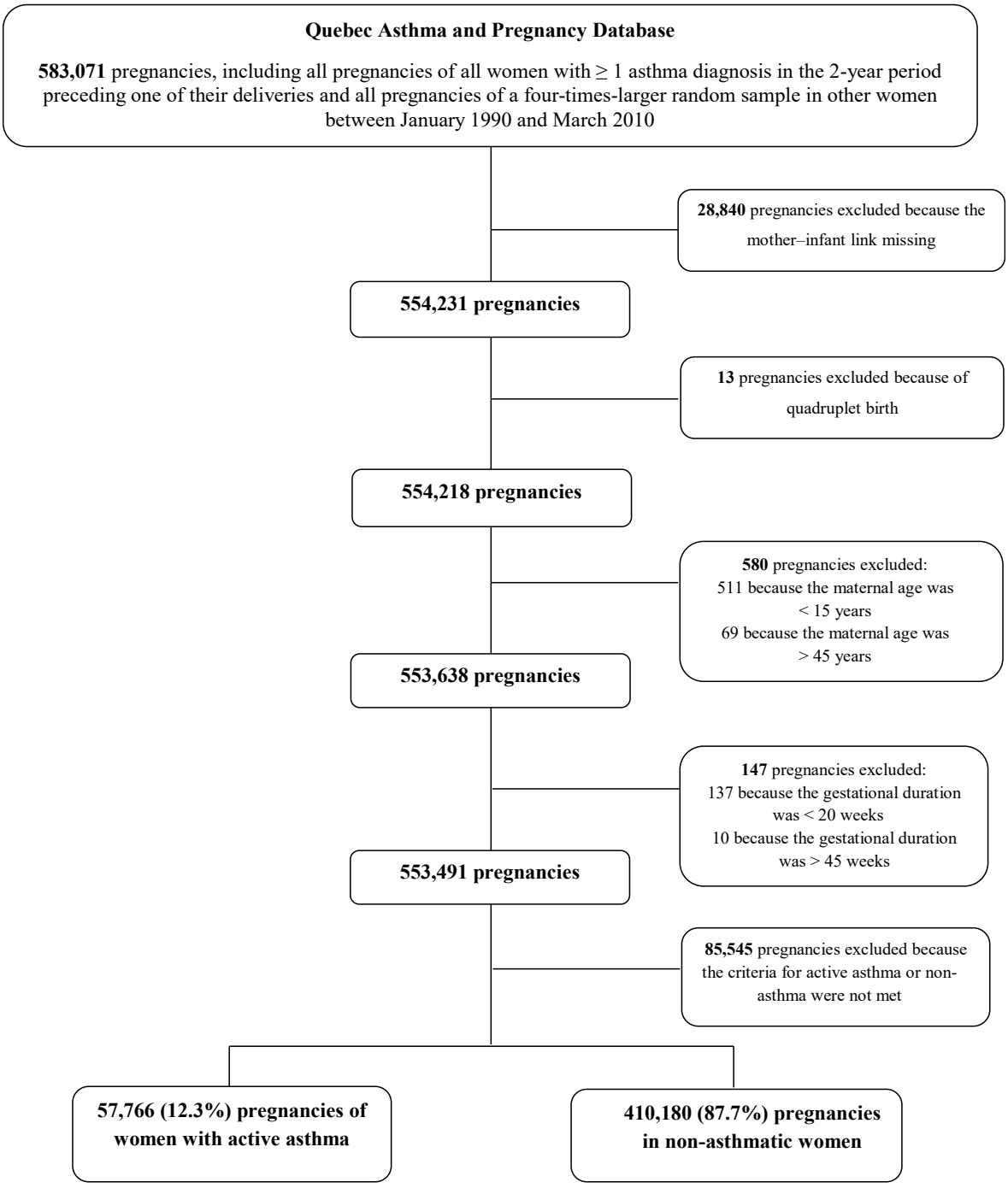

Figure 1. Cohort selection flow-diagram

Table 1. Case Ascertainment Definitions for the Major Congenital Malformations Used in This Study

Case ascertainment definition	**Data source**		
	Hospitalization database (MED-ECHO)		Medical claims database (RAMQ)
TCMC			
≥ 1 hospitalization	≥ 1 diagnostic code of major malformation		
≥ 1 hospitalization or ≥ 2 medical claims	≥ 1 diagnostic code of major malformation	or	≥ 2 diagnostic codes of major malformation
≥ 1 hospitalization or ≥ 1 medical claim	≥ 1 diagnostic code of major malformation	or	≥ 1 diagnostic code of major malformation
CCASS			
≥ 1 hospitalization	≥ 1 diagnostic code of major malformation		
≥ 1 hospitalization or ≥ 2 medical claims	≥ 1 diagnostic code of major malformation	or	≥ 2 diagnostic codes of major malformation
≥ 1 hospitalization or ≥ 1 medical claim	≥ 1 diagnostic code of major malformation	or	≥ 1 diagnostic code of major malformation

Abbreviations: MED-ECHO: Maintenance et Exploitation des Données pour l'Étude de la Clientèle Hospitalière; RAMQ: Régie de l'Assurance-Maladie du Québec; TCMC: Two step Congenital Malformation Classification; CCASS: Canadian Congenital Anomalies Surveillance System classification.

Table 2. Characteristics of the Pregnancies of Women With Active Asthma and Non-asthmatic Women

	Pregnancies of women with active asthma (*n* = 57,766)		**Pregnancies in non-asthmatic women (*n* = 410,180)**	
	No.	%	No.	%
Maternal age at the start of pregnancy (years)				
< 18	1,827	3.2	6,089	1.5
18–34	50,643	87.7	362,548	88.4
≥ 35	5,296	9.2	41,543	10.1
Receipt of social assistance at the start of pregnancy	10,510	18.2	40,490	9.9
Urban area of residence at delivery	47,863	82.9	323,578	78.9
Multiple pregnancy	890	1.5	5,152	1.3
Diagnoses before pregnancy				
Chronic hypertension	4,862	8.4	20,634	5.0
Diabetes mellitus	5,898	10.2	31,045	7.6
Epilepsy	342	0.6	1,316	0.3

Table 3. Prevalence of Major Congenital Malformations According to the Classification Method and Case Ascertainment Definition

	TCMC classification						CCASS classification					
	≥ 1 hospitalization		≥ 1 hospitalization or ≥ 2 medical claims		≥ 1 hospitalization or ≥ 1 medical claim		≥ 1 hospitalization		≥ 1 hospitalization or ≥ 2 medical claims		≥ 1 hospitalization or ≥ 1 medical claim	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Any major malformation [a]	23,868	5.1	26,273	5.6	33,142	7.1	32,894	7.0	37,396	8.0	49,439	10.6
System-specific malformations												
Neural tube defects	191	0.0	224	0.1	314	0.1	191	0.0	224	0.1	314	0.1
Central nervous system [b]	1,252	0.3	1,508	0.3	2,287	0.5	1,252	0.3	1,508	0.3	2,287	0.5
Cardiac	4,792	1.0	5,870	1.3	8,191	1.8	4,792	1.0	5,870	1.3	8,191	1.8
Circulatory system	1,304	0.3	1,572	0.3	2,070	0.4	3,145	0.7	3,440	0.7	4,059	0.9
Respiratory system	1,206	0.3	1,224	0.3	1,330	0.3	580	0.1	723	0.2	1,205	0.3
Eye	603	0.1	856	0.2	2,416	0.5	304	0.1	566	0.1	2,147	0.5
Ear, face, and neck	124	0.0	128	0.0	146	0.0	1,684	0.4	1,769	0.4	2,163	0.5
Orofacial clefts	599	0.1	627	0.1	697	0.2	599	0.1	627	0.1	697	0.2
Digestive system	3,591	0.8	3,859	0.8	4,772	1.0	1,901	0.4	2,119	0.5	2,622	0.6
Genital organs	898	0.2	1,100	0.2	1,480	0.3	2,577	0.6	3,052	0.7	4,255	0.9

Urinary system	3,395	0.7	3,463	0.7	3,654	0.8	3,367	0.7	3,436	0.7	3,622	0.8
Limb and musculoskeletal	5,002	1.1	5,279	1.1	5,889	1.3	15,143	3.2	16,980	3.6	21,317	4.6
Integument	3,210	0.7	3,226	0.7	3,316	0.7	1,070	0.2	1,104	0.2	1,392	0.3
Down syndrome	494	0.1	513	0.1	560	0.1	494	0.1	513	0.1	560	0.1
Other chromosomal	269	0.1	601	0.1	1,193	0.3	207	0.0	211	0.1	223	0.1
Other and unspecified	478	0.1	515	0.1	592	0.1	984	0.2	2,366	0.5	4,813	1.0

Abbreviations: TCMC: Two step Congenital Malformation Classification method; CCASS: Canadian Congenital Anomalies Surveillance System classification method; ≥ 1 hospitalization: ≥ 1 major malformation diagnosis recorded in the hospital database; ≥ 1 hospitalization or ≥ 2 medical claims: ≥ 2 major malformation diagnoses recorded in the medical claims database or ≥ 1 major malformation diagnosis recorded in the hospital database; ≥ 1 hospitalization or ≥ 1 medical claim: ≥ 1 major malformation diagnoses recorded in the medical claims database or ≥ 1 major malformation diagnosis recorded in the hospital database.

[a] The sum of all system-specific malformations in each column exceeds the total number of cases with any major malformation because some infants had multiple malformations.

[b] Includes neural tube defects.

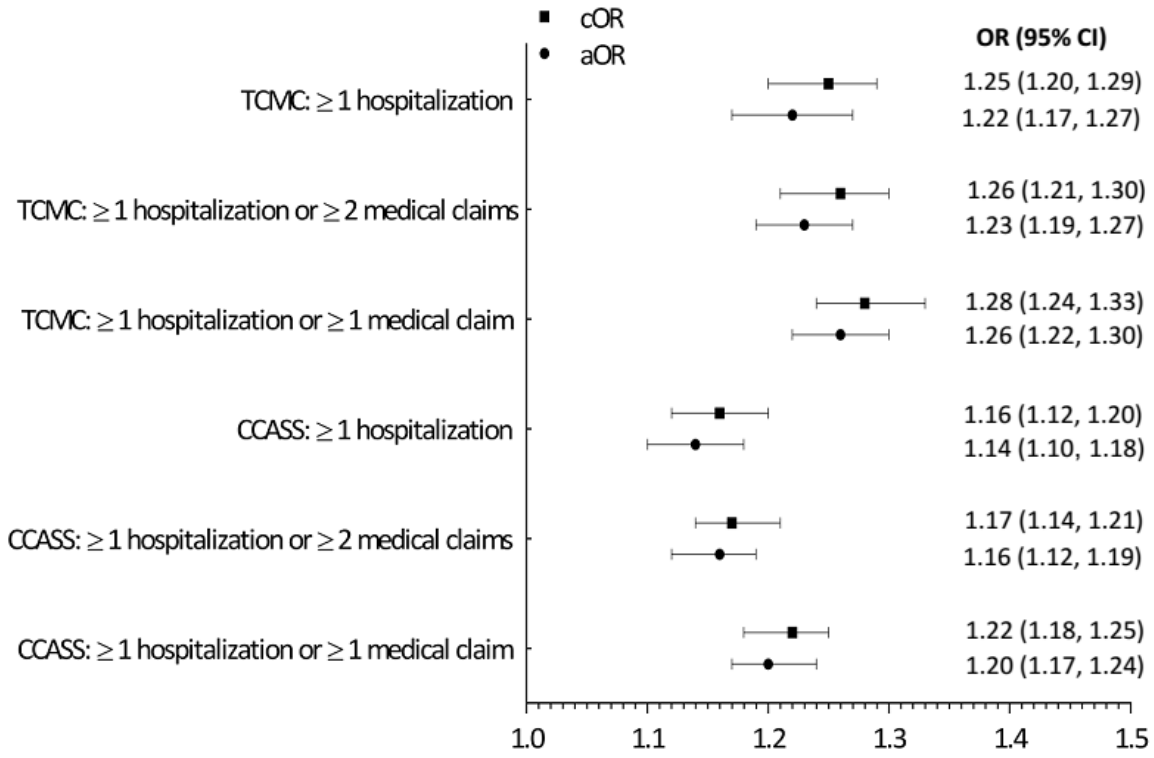

Figure 2. Crude and adjusted odds ratios for major malformations in the pregnancies of women with active asthma versus pregnancies in non-asthmatic women using the specified case ascertainment definitions

Abbreviations: TCMC: Two step Congenital Malformation Classification method; CCASS: Canadian Congenital Anomalies Surveillance System classification method; ≥ 1 hospitalization: ≥ 1 major malformation diagnosis recorded in the hospital database; ≥ 1 hospitalization or ≥ 2 medical claims: ≥ 2 major malformation diagnoses recorded in the medical claims database or ≥ 1 major malformation diagnosis recorded in the hospital database; ≥ 1 hospitalization or ≥ 1 medical claim: ≥ 1 major malformation diagnoses recorded in the medical claims database or ≥ 1 major malformation diagnosis recorded in the hospital database; cOR: crude odds ratio; aOR: adjusted odds ratio; OR: odds ratio; CI: confidence interval.

Table e1. Congenital Malformation Classification Systems and Related ICD-9 Codes*

	TCMC classification			CCASS classification	
	Major	**Major/Minor†**	**Minor (Excluded)**	**Major**	**Minor (Excluded)**
Specific malformations	**ICD-9 codes**				
Nervous system‡	740: Anencephalus and similar anomalies 741: Spina bifida 742: Other congenital anomalies of nervous system			740: Anencephalus and similar anomalies 741: Spina bifida 742: Other congenital anomalies of nervous system	
Eye	743.0: Anophthalmos 743.1: Microphthalmos 743.2: Buphthalmos 743.3: Congenital cataract and lens anomalies 743.4: Coloboma and other anomalies of anterior segment 743.5: Congenital anomalies of posterior segment 743.8: other specified anomalies of eye 743.9: Unspecified anomaly of eye	743.6: Congenital anomalies of eyelids, lacrimal system, and orbit		743.0: Anophthalmos 743.1: Microphthalmos 743.2: Buphthalmos 743.3: Congenital cataract and lens anomalies 743.4: Coloboma and other anomalies of anterior segment 743.5: Congenital anomalies of posterior segment 743.8: other specified anomalies of eye 743.9: Unspecified anomaly of eye	743.6: Congenital anomalies of eyelids, lacrimal system, and orbit
Ear, face and neck	744.0: Anomalies of ear causing impairment of hearing	744.3:Unspecified congenital anomaly of ear 744.4: Branchial cleft cyst or fistula; preauricular sinus 744.9: Unspecified congenital anomalies of face and neck	744.1: Accessory auricle 744.2: Other specified congenital anomalies of ear 744.5: Webbing of neck 744.8: Other	744.0: Anomalies of ear causing impairment of hearing 744.1: Accessory auricle 744.2: Other specified congenital anomalies of ear 744.3:Unspecified congenital anomaly of ear 744.4: Branchial cleft cyst or fistula; preauricular sinus	

	TCMC classification			CCASS classification	
	Major	**Major/Minor†**	**Minor (Excluded)**	**Major**	**Minor (Excluded)**
			specified congenital anomalies of face and neck	744.5: Webbing of neck 744.8: Other specified congenital anomalies of face and neck 744.9: Unspecified congenital anomalies of face and neck	
Cardiac	745: Bulbus cordis anomalies and anomalies of cardiac septal closure 746: Other congenital anomalies of heart			745: Bulbus cordis anomalies and anomalies of cardiac septal closure 746: Other congenital anomalies of heart	
Circulatory system	747.1: Coarctation of aorta 747.2: Other anomalies of aorta 747.3: Anomalies of pulmonary artery 747.4: Anomalies of great veins 747.6: Other anomalies Of peripheral vascular system 747.8: Other specified anomalies of circulatory system 747.9: Unspecified anomaly of circulatory system	747.0: Patent ductus arteriosus	747.5: Absence or hypoplasia of umbilical artery	747.0: Patent ductus arteriosus 747.1: Coarctation of aorta 747.2: Other anomalies of aorta 747.3: Anomalies of pulmonary artery 747.4: Anomalies of great veins 747.5: Absence or hypoplasia of umbilical artery 747.6: Other anomalies Of peripheral vascular system 747.8: Other specified anomalies of circulatory system 747.9: Unspecified anomaly of circulatory system	
Respiratory system	748.1: Other anomalies of nose 748.2: Web of larynx 748.3: Other anomalies of larynx, trachea, and bronchus 748.4: Congenital cystic lung 748.5: Agenesis, hypoplasia, and dysplasia of lung	748.0: Choanal atresia 748.9: Unspecified anomaly of respiratory system		748.0: Choanal atresia 748.1: Other anomalies of nose 748.2: Web of larynx 748.4: Congenital cystic lung 748.5: Agenesis, hypoplasia, and dysplasia of lung 748.6: Other anomalies of lung	748.3: Other anomalies of larynx, trachea, and bronchus

	TCMC classification			CCASS classification	
	Major	**Major/Minor†**	**Minor (Excluded)**	**Major**	**Minor (Excluded)**
	748.6: Other anomalies of lung 748.8: Other specified anomalies of respiratory system			748.8: Other specified anomalies of respiratory system 748.9: Unspecified anomaly of respiratory system	
Cleft palate and cleft lip	749: Cleft palate and cleft lip			749: Cleft palate and cleft lip	

	TCMC classification			CCASS classification	
	Major	**Major/Minor†**	**Minor (Excluded)**	**Major**	**Minor (Excluded)**
Digestive system	750.0: Tongue tie 750.3: Tracheoesophageal fistula, esophageal atresia and stenosis 750.4: Other specified anomalies of esophagus 750.5: Congenital hypertrophic pyloric stenosis 750.6: Congenital hiatus hernia 750.7: Other specified anomalies of stomach 750.8: Other specified anomalies of upper alimentary tract 751.0: Meckel's diverticulum 751.1: Atresia and stenosis of small intestine 751.2: Atresia and stenosis of large intestine, rectum, and anal canal 751.3: Hirschsprung's disease and other congenital functional disorders of colon 751.4: Anomalies of intestinal fixation 751.5: Other anomalies of intestine 751.6: Anomalies of gallbladder, bile ducts, and liver 751.7: Anomalies of pancreas 751.8: Other specified anomalies of digestive system	750.1: Other anomalies of tongue 750.2: Other specified congenital anomalies of mouth and pharynx 750.9: Unspecified anomaly of upper alimentary tract 751.9: Unspecified anomaly of digestive system		750.1: Other Anomalies Of Tongue 750.2: Other specified congenital anomalies of mouth and pharynx 750.3: Tracheoesophageal fistula, esophageal atresia and stenosis 750.4: Other specified anomalies of esophagus 750.5: Congenital hypertrophic pyloric stenosis 750.6: Congenital hiatus hernia 750.7: Other specified anomalies of stomach 750.8: Other specified anomalies of upper alimentary tract 750.9: Unspecified anomaly of upper alimentary tract 751.0: Meckel's diverticulum 751.1: Atresia and stenosis of small intestine 751.2: Atresia and stenosis of large intestine, rectum, and anal canal 751.3: Hirschsprung's disease and other congenital functional disorders of colon 751.4: Anomalies of intestinal fixation 751.6: Anomalies of gallbladder, bile ducts, and liver 751.7: Anomalies of pancreas 751.8: Other specified anomalies of digestive system 751.9: Unspecified anomaly of digestive system	750.0: Tongue tie 751.5: Other anomalies of intestine

	TCMC classification			CCASS classification	
	Major	**Major/Minor†**	**Minor (Excluded)**	**Major**	**Minor (Excluded)**
Genital organs	752.0: Anomalies of ovaries 752.1: Anomalies of fallopian tubes and broad ligaments 752.2: Doubling of uterus 752.3: Other anomalies of uterus 752.4: Anomalies of cervix, vagina, and external female genitalia 752.7: Indeterminate sex and pseudohermaphroditism	752.5:Undescended and retractile testicle 752.6: Hypospadias and epispadias and other penile anomalies 752.8: Other specified congenital anomalies of genital organs 752.9: Unspecified anomaly of genital organs		752.0: Anomalies of ovaries 752.1: Anomalies of fallopian tubes and broad ligaments 752.2: Doubling of uterus 752.3: Other anomalies of uterus 752.4: Anomalies of cervix, vagina, and external female genitalia 752.6: Hypospadias and epispadias and other penile anomalies 752.7: Indeterminate sex and pseudohermaphroditism 752.8: Other specified anomalies of genital organs 752.9: Unspecified anomaly of genital organs	752.5: Undescended and retractile testicle

	TCMC classification			CCASS classification	
	Major	**Major/Minor†**	**Minor (Excluded)**	**Major**	**Minor (Excluded)**
Urinary system	753.0: Renal agenesis and dysgenesis 753.1: Cystic kidney disease 753.2: Obstructive defects of renal pelvis and ureter 753.3: Other specified anomalies of kidney 753.4: Other specified anomalies of ureter 753.5: Exstrophy of urinary bladder 753.6: Atresia and stenosis of urethra and bladder neck 753.7: Anomalies of urachus 753.8: Other specified anomalies of bladder and urethra 753.9: Unspecified anomaly of urinary system			753.0: Renal agenesis and dysgenesis 753.1: Cystic kidney disease 753.2: Obstructive defects of renal pelvis and ureter 753.3: Other specified anomalies of kidney 753.4: Other specified anomalies of ureter 753.5: Exstrophy of urinary bladder 753.7: Anomalies of urachus 753.8: Other specified anomalies of bladder and urethra 753.9: Unspecified anomaly of urinary system	753.6: Atresia and stenosis of urethra and bladder neck
Limb & Musculoskeletal	754.1: Congenital musculoskeletal deformities of sternocleidomastoid muscle 754.2: Congenital musculoskeletal deformities of spine 754.3: Congenital dislocation of hip 754.4: Congenital genu recurvatum and bowing of long bones of leg 755.2: Reduction deformities of upper limb congenital 755.3: Congenital reduction deformities of lower limb 755.4: Reduction deformities,	754.0: Of skull, face, and jaw 754.5: Varus deformities of feet 754.6: Valgus deformities of feet 754.7: Other deformities of feet 754.8: Other specified nonteratogenic anomalies 754.9: unspecified 755.0: Polydactyly 755.1: Syndactyly		754.1: Of sternocleidomastoid muscle 754.2: Of spine 754.3: Congenital dislocation of hip 754.4: Congenital genu recurvatum and bowing of long bones of leg 754.5: Varus deformities of feet 754.6: Valgus deformities of feet 754.7: Other deformities of feet 754.8: Other specified nonteratogenic anomalies 755.0: Polydactyly 755.1: Syndactyly	754.0: Congenital musculoskeletal deformities of skull, face, and jaw 754.9: unspecified

TCMC classification			CCASS classification	
Major	**Major/Minor†**	**Minor (Excluded)**	**Major**	**Minor (Excluded)**
unspecified limb 756.4: Chondrodystrophy 756.5: Congenital osteodystrophies 756.7: Congenital anomalies of abdominal wall 756.8: Other specified congenital anomalies of muscle tendon fascia and connective tissue	755.5: Other anomalies of upper limb, including shoulder girdle 755.6: Other anomalies of lower limb, including pelvic girdle 755.8: Other specified anomalies of unspecified limb 755.9: Unspecified anomaly of unspecified limb 756.0: Anomalies of skull and face bones 756.1: Anomalies of spine 756.2: Cervical rib 756.3: Other anomalies of ribs and sternum 756.6: Anomalies of diaphragm 756.9: Other and unspecified anomalies of musculoskeletal system.		755.2: Reduction deformities of upper limb 755.3: Reduction deformities of lower limb 755.4: Reduction deformities, unspecified limb 755.5: Other anomalies of upper limb, including shoulder girdle 755.6: Other anomalies of lower limb, including pelvic girdle 755.8: Other specified anomalies of unspecified limb 755.9: Unspecified anomaly of unspecified limb 756.0: Anomalies of skull and face bones 756.1: Anomalies of spine 756.2: Cervical rib 756.3: Other anomalies of ribs and sternum 756.4: Chondrodystrophy 756.5: Osteodystrophies 756.6: Anomalies of diaphragm 756.7: Anomalies of abdominal wall 756.8: Other specified anomalies of muscle, tendon, fascia, and connective tissue 756.9: Other and unspecified anomalies of musculoskeletal system	

	TCMC classification			CCASS classification	
	Major	**Major/Minor†**	**Minor (Excluded)**	**Major**	**Minor (Excluded)**
Integument	757.1: Ichthyosis congenita 757.3: Other specified congenital anomalies of skin	757.0: Hereditary edema of legs 757.4: Specified anomalies of hair 757.5: Specified anomalies of nails 757.6: Specified anomalies of breast 757.8: Other specified anomalies of the integument 757.9: Unspecified anomaly of the integument	757.2: Dermatoglyphic anomalies	757.0: Hereditary edema of legs 757.1: Ichthyosis congenita 757.2: Dermatoglyphic anomalies 757.4: Specified anomalies of hair 757.5: Specified anomalies of nails 757.6: Specified anomalies of breast 757.8: Other specified anomalies of the integument 757.9: Unspecified anomaly of the integument	757.3: Other specified congenital anomalies of skin
Chromosomal§	758.0: Down syndrome 758.1: Patau's syndrome 758.2: Edward's syndrome 758.3: Autosomal deletion syndromes 758.4: Balanced autosomal translocation in normal individual 758.5: Other conditions due to autosomal anomalies 758.6: Gonadal dysgenesis 758.7: Klinefelter's syndrome 758.8: Other conditions due to chromosome anomalies 758.9: Conditions due to anomaly of unspecified chromosome			758.0: Down syndrome 758.1: Patau's syndrome 758.2: Edward's syndrome 758.3: Autosomal deletion syndromes 758.4: Balanced autosomal translocation in normal individual 758.5: Other conditions due to autosomal anomalies 758.6: Gonadal dysgenesis 758.7: Klinefelter's syndrome 758.8: Other conditions due to chromosome anomalies	

	TCMC classification			CCASS classification	
	Major	**Major/Minor**[†]	**Minor (Excluded)**	**Major**	**Minor (Excluded)**
Other congenital malformations	759.1: Anomalies of adrenal gland 759.3: Situs inversus 759.4: Conjoined twins 759.5: Tuberous sclerosis 759.6: Other hamartoses, NEC 759.7: Multiple congenital anomalies, so described	759.0: Anomalies of spleen 759.2: Anomalies of other endocrine glands 759.8: Other specified congenital anomalies 759.9: Congenital anomaly, unspecified		758.9: Conditions due to anomaly of unspecified chromosome 759.0: Anomalies of spleen 759.1: Anomalies of adrenal gland 759.2: Anomalies of other endocrine glands 759.3: Situs inversus 759.4: Conjoined twins 759.5: Tuberous sclerosis 759.6: Other hamartoses, NEC 759.7: Multiple congenital anomalies, so described 759.8: Other specified anomalies 759.9: Congenital anomaly, unspecified	

*For congenital malformations recorded in the *Maintenance et Exploitation des Données pour l'Étude de la Clientèle Hospitalière* (MED-ECHO) hospitalization database, comparable codes from the enhanced version of the International Classification of Diseases (ICD) 10th revision for Canada (ICD-10-CA) were used since 2006.

[†]When a malformation was classified as major or minor by the geneticist, it was only recorded as a major malformation if it was associated with at least one hospitalization with a primary or an admission diagnosis related to this malformation during the infant's 1st year of life.

[‡] Neural tube defects were reported separately.

[§] Down syndrome was reported separately.

TCMC: Two step Congenital Malformation Classification method; *CCASS*: Canadian Congenital Anomalies Surveillance System classification method; NEC: not elsewhere classified.

Table e2. Crude and Adjusted Odds Ratios for Major Malformations in the Pregnancies of Women With Active Asthma Versus Pregnancies in Non-asthmatic Women Using the Specified Case Ascertainment Definitions [a]

Case ascertainment definition	Asthma	No. of pregnancies	No. of cases of major malformation (%)	Crude OR (95% CI)	Adjusted OR (95% CI)
TCMC					
≥ 1 hospitalization	Yes	57,766	3,532 (6.1)	1.25 (1.20, 1.29)	1.22 (1.17, 1.27)
	No	410,180	20,336 (5.0)	Reference	Reference
≥ 1 hospitalization or ≥ 2 medical claims	Yes	57,766	3,903 (6.8)	1.26 (1.21, 1.30)	1.23 (1.19, 1.27)
	No	410,180	22,370 (5.5)	Reference	Reference
≥ 1 hospitalization or ≥ 1 medical claim	Yes	57,766	4,998 (8.7)	1.28 (1.24, 1.33)	1.26 (1.22, 1.30)
	No	410,180	28,144 (6.9)	Reference	Reference
CCASS					
≥ 1 hospitalization	Yes	57,766	4,570 (7.9)	1.16 (1.12, 1.20)	1.14 (1.10, 1.18)
	No	410,180	28,324 (6.9)	Reference	Reference
≥ 1 hospitalization or ≥ 2 medical claims	Yes	57,766	5,248 (9.1)	1.17 (1.14, 1.21)	1.16 (1.12, 1.19)
	No	410,180	32,148 (7.8)	Reference	Reference
≥ 1 hospitalization or ≥ 1 medical claim	Yes	57,766	7,100 (12.3)	1.22 (1.18, 1.25)	1.20 (1.17, 1.24)
	No	410,180	42,339 (10.3)	Reference	Reference

[a] Generalized estimating equation models were used with exchangeable correlation matrix

Abbreviations: TCMC: Two step Congenital Malformation Classification method; CCASS: Canadian Congenital Anomalies Surveillance System classification method; ≥ 1 hospitalization: ≥ 1 major malformation diagnosis recorded in the hospital database; ≥ 1 hospitalization or ≥ 2 medical claims: ≥ 2 major malformation diagnoses recorded in the medical claims database or ≥ 1 major malformation diagnosis recorded in the hospital database; ≥ 1 hospitalization or ≥ 1 medical claim: ≥ 1 major malformation diagnoses recorded in the medical claims database or ≥ 1 major malformation diagnosis recorded in the hospital database; OR: odds ratio; CI: confidence interval.

5.3.2 Unpublished sensitivity analysis

The results of the GEE models according to the correlation matrix used are presented in Table 5.3.2. The results obtained using the independent correlation matrix were consistent with those obtained with the exchangeable matrix. However, when the unstructured correlation matrix was used, the CCASS method yielded inconsistent and imprecise results, mainly due to convergence issues. These inconsistencies and imprecisions were not observed when we used the TCMC method.

Table 5.3.2 Crude and Adjusted Odds Ratios for Major Malformations in the Pregnancies of Women with Active Asthma versus Pregnancies in Non-asthmatic Women Using the Specified Case Ascertainment Definitions and Correlation Matrices

Case ascertainment definition	Correlation matrix	Crude OR (95% CI)	Adjusted OR (95% CI)
TCMC			
≥ 1 hospitalization	Unstructured	1.25 (1.20, 1.29)	1.22 (1.17, 1.27)
	Exchangeable	1.25 (1.20, 1.29)	1.22 (1.17, 1.27)
	Independent	1.25 (1.20, 1.30)	1.22 (1.17, 1.27)
≥ 1 hospitalization or ≥ 2 medical claims	Unstructured	1.26 (1.21, 1.30)	1.23 (1.19, 1.27)
	Exchangeable	1.26 (1.21, 1.30)	1.23 (1.19, 1.27)
	Independent	1.26 (1.21, 1.30)	1.23 (1.19, 1.27)
≥ 1 hospitalization or ≥ 1 medical claim	Unstructured	1.28 (1.24, 1.33)	1.26 (1.22, 1.30)
	Exchangeable	1.28 (1.24, 1.33)	1.26 (1.22, 1.30)
	Independent	1.29 (1.25, 1.33)	1.26 (1.22, 1.30)
CCASS			
≥ 1 hospitalization	Unstructured	1.16 (0.82, 1.64)	1.09 (0.82, 1.47)
	Exchangeable	1.16 (1.12, 1.20)	1.14 (1.10, 1.18)
	Independent	1.16 (1.12, 1.20)	1.14 (1.10, 1.18)
≥ 1 hospitalization or ≥ 2 medical claims	Unstructured	1.22 (0.89, 1.66)	1.16 (0.90, 1.49)
	Exchangeable	1.17 (1.14, 1.21)	1.16 (1.12, 1.19)
	Independent	1.18 (1.14, 1.21)	1.16 (1.12, 1.19)
≥ 1 hospitalization or ≥ 1 medical claim	Unstructured	1.31 (1.06, 1.61)	1.27 (1.09, 1.46)
	Exchangeable	1.22 (1.18, 1.25)	1.20 (1.17, 1.24)
	Independent	1.22 (1.18, 1.25)	1.20 (1.17, 1.24)

Abbreviations: TCMC: Two step Congenital Malformation Classification method; CCASS: Canadian Congenital Anomalies Surveillance System classification method; ≥ 1 hospitalization: ≥ 1 major malformation diagnosis recorded in the hospital database; ≥ 1 hospitalization or ≥ 2 medical claims: ≥ 2 major malformation diagnoses recorded in the medical claims database or ≥ 1 major malformation diagnosis recorded in the hospital database; ≥ 1 hospitalization or ≥ 1 medical claim: ≥ 1 major malformation diagnoses recorded in the medical claims database or ≥ 1 major malformation diagnosis recorded in the hospital database; OR: odds ratio; CI: confidence interval.

5.4 Article on the systematic procedure for the classification of proven and potential teratogens

Systematic Procedure for the Classification of Proven and Potential Teratogens for Use in Research

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Systematic Procedure for the Classification of Proven and Potential Teratogens for Use in Research

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Short title: Systematic procedure for teratogens classification

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ABSTRACT

Background: Although there is strong evidence that some medications are teratogenic, the current lists of teratogens to be used in research are outdated.

Objectives: To develop an updatable and systematic procedure to the classification of medications proven and potentially teratogenic in the first trimester of pregnancy, for use in research.

Methods: We developed a two-step procedure for teratogen classification. Step 1 includes classifying the medications from *Drugs in Pregnancy and Lactation: a Reference Guide to Fetal and Neonatal Risk* (9th ed.) into two provisional lists: 1) teratogenic medications, and 2) potentially teratogenic medications. We also searched other references to add other medications. In Step 2, the Teratology Information System (TERIS) database was searched, and the medication was classified as teratogenic or potentially teratogenic according to a newly developed scheme. Expert consensus was used if a medication was not recorded in TERIS.

Results: 114 medications were identified in *Drugs in Pregnancy and Lactation: a Reference Guide to Fetal and Neonatal Risk*, with 57 medications in each provisional list. 78 medications were identified in other sources. 135 medications were included in Step 2; the TERIS scheme classified 23 medications, and 112 medications required expert opinion. The two experts agreed on 78.6% of the medications (kappa = 0.63). We identified 91 teratogenic and 81 potentially teratogenic medications.

Conclusions: Using reliable references, we established a systematic procedure to the classification of medications with evidence of or potential teratogenic risk. These exhaustive lists will be useful in teratology research and related fields.

Key words: Teratogen, medication, epidemiology, birth defects, first trimester

INTRODUCTION

Pregnant women living in developed countries frequently take prescription and over-the-counter (OTC) medications, with prevalence estimates ranging between 27% and 99%, depending on the medications examined and the data sources used.(Daw and others, 2012; Friedman, 2012; Mitchell and others, 2011) Moreover, the numbers of women taking medications during pregnancy is growing as maternal age increases, and with the increasing use of medications in developed countries.(Mazer-Amirshahi and others, 2014; Shahin and Einarson, 2011; Thorpe and others, 2013; Wysowski and others, 2006) Maternal exposure to medications during pregnancy is often unavoidable because they are used to treat chronic diseases or because the pregnancy was not yet recognized.(Mazer-Amirshahi and others, 2014; Mitchell and others, 2011) A Dutch report showed that 17.5% of women took a suspected teratogenic medication during the first trimester of pregnancy,(van Gelder and others, 2014a) and in the United States, it was reported that 23% of the medications most commonly used during the first trimester were included in Category X (risks involved in use of the drug clearly outweigh potential benefits.).(Thorpe and others, 2013)

Since the tragedy of thalidomide, the teratogenic effects of a number of medications, acting through various mechanisms, have been demonstrated.(Adam and others, 2011; Briggs and others, 2011; Buonocore and others, 2010; Ferreira and others, 2013; Friedman, 2012; Obican and Scialli, 2011; van Gelder and others, 2014b) However, there are scarce data on the majority of the medications used by pregnant women to assess their potential teratogenic risk in humans.(Friedman, 2012; Thorpe and others, 2013) A published report in 2011, based on expert reviews by the Teratology Information System (TERIS), showed that among 172 medications approved in the United States between 2000 and 2010, 97.7% had insufficient published data and 73.3% had no human data with which to determine their teratogenic risk in humans.(Adam and others, 2011) However, new evidence is constantly produced for currently marketed medications, and several information sources can be accessed for a better assessment of teratogenic risk.(Adam and others, 2011; Briggs and others, 2015; REPROTOX®; Schaefer and others, 2015; Shepard, 2010; Teratogen

Information System (TERIS)) Observational studies are being increasingly used in assessing the teratogenic risk of medications used during pregnancy. Despite their benefits, which include the large sample sizes and low costs, observational studies incur significant validity threats. When observational data are used to study a teratogenic drug effect, it is essential to control for important risk factors for congenital malformations and most importantly, maternal exposure to other potential teratogens. Several databases and references on teratogenic risks are currently available, providing either complete or partial evidence for the teratogenicity of medications.(Banhidy and others, 2005; Buonocore and others, 2010; Goodwin, 2010; Kalter, 2010; Koren and others, 1998; Malm and others, 2004; Polifka and Friedman, 2002; Porter and others, 2006; Queenan and others, 2010; Schaefer and others, 2015; Seyberth and others, 2011; Stevenson, 2006; Webster and Freeman, 2003) However, there are substantial discrepancies between the lists of medications that should be considered teratogenic, and significant imprecision is added when categories are used (e.g., moderate- vs high-risk teratogens).(Banhidy and others, 2005; Buonocore and others, 2010; Goodwin, 2010; Kalter, 2010; Koren and others, 1998; Malm and others, 2004; Polifka and Friedman, 2002; Porter and others, 2006; Queenan and others, 2010; Seyberth and others, 2011; Stevenson, 2006; Webster and Freeman, 2003) Therefore, harnessing the full potential of several reliable resources is essential to the creation of a comprehensive overview.

No updatable systematic procedure to classify medications into proven and potential teratogens exists, and currently available lists are outdated on several levels.(Buonocore and others, 2010; Kalter, 2010; Porter and others, 2006; Queenan and others, 2010; Seyberth and others, 2011) Therefore, based on the whole corpus of leading teratology resources, we aimed to develop a systematic and updatable procedure for the classification of medications into those with sufficient evidence of teratogenic risk and those with potential teratogenic risk during the first trimester of pregnancy for use in research. The lists should be used only for research and not for clinical or counseling purposes.

METHODS

Steps and settings

We developed a systematic two-step procedure for teratogen identification and classification. The goal was to construct two medication lists, one including "medications with sufficient evidence of a teratogenic risk in the first trimester", hereinafter referred to as "teratogenic medications", and the other including "medications with a potential teratogenic risk in the first trimester based on human and/or animal data", hereinafter referred to as "potentially teratogenic medications". The first trimester was considered the period of interest in our classification to maintain the reliability and validity of the results.

Step 1, presented in Figure 1, included the identification and classification of medications reported in the reference book *Drugs in Pregnancy and Lactation: a Reference Guide to Fetal and Neonatal Risk* (9th ed.) by Briggs et al. 2011 (Briggs and others, 2011) into two provisional lists: 1) teratogenic medications, and 2) potentially teratogenic medications. Briggs et al. (2011) is a well-known reference that offers a careful and exhaustive summary of the world literature relating to drugs administered during pregnancy and lactation.(Briggs and others, 2011)

Provisional List 1 (teratogenic medications) included all the medications in Briggs et al. (2011) listed under the pregnancy recommendations as "contraindicated—1st trimester" and "contraindicated". Provisional List 2 (potentially teratogenic medications) included all the medications in Briggs et al. (2011) listed under the pregnancy recommendations as "human data suggest risk in 1st and 3rd trimesters", "human (and animal) data suggest risk", "no (limited) human data—animal data suggest risk", "no (limited) human data—animal data suggest moderate risk", and "no (limited) human data—animal data suggest high risk".(Briggs and others, 2011) These provisional lists were then verified by a teratology expert (B.M.) for the accuracy of the classification and inclusion into teratogens or potential teratogens lists, leading to either the approval of classification (agreement upon the classification of the medications in the Final lists) or further verification, i.e., entry on a "verification list". We also searched other references, including reviews of teratogenic drugs and drug-related birth defects, textbooks of teratogenicity, and Briggs et al. updates (till October 2013, the latest in our possession), to identify other potential teratogens to be added to the verification list.(Brent, 2004; Buonocore and others, 2010; Goodwin, 2010; Kalter, 2010; Koren and others, 1998; Malm and others, 2004; Polifka and Friedman, 2002;

Porter and others, 2006; Queenan and others, 2010; Seyberth and others, 2011; Stevenson, 2006) The medications cited in Briggs et al. updates were considered if they met any of the pregnancy recommendations mentioned previously for provisional Lists 1 and 2, whereas all possible teratogens reported in the other references were included in the verification list.(Brent, 2004; Buonocore and others, 2010; Goodwin, 2010; Kalter, 2010; Koren and others, 1998; Malm and others, 2004; Polifka and Friedman, 2002; Porter and others, 2006; Queenan and others, 2010; Seyberth and others, 2011; Stevenson, 2006)

In Step 2, we searched the TERIS database for each medication included in the verification list. TERIS is an online clinical teratology resource.(Adam and others, 2011) It is supported by an expert advisory board, which assigns a teratogenicity risk rating to each medication included in the TERIS database. As new evidence of the teratogenicity of a medication becomes available, the risk ratings are updated.(Adam and others, 2011) Each adviser on the board independently rates the quality and quantity of data for each medication and the magnitude of the teratogenic risk it carries. The quality and quantity of data are classified as either “none”, “very limited”, “limited”, “fair”, “good”, or “excellent”, with ratings intermediate between two of these in some cases (e.g., good to excellent). The magnitude of the teratogenic risk is described as either “none”, “unlikely”, “minimal”, “small”, “moderate”, “high”, or “undetermined”. The medication ratings by the advisory board are developed through consensus after a thorough examination of the published data available on the medications from several sources.(Adam and others, 2011) We searched Briggs et al. (2011) first and then used the TERIS database for verification and classification in Step 2– rather than being searched for potential teratogenic medications–for two reasons. First, Briggs et al. is the more comprehensive reference, including a larger number of medications than TERIS with an online index searchable by the medications’ pregnancy recommendations. Second, some agents have more than one magnitude of teratogenic risk rating in TERIS database and it is currently not possible to do a search using the "quality & quantity of data" ratings, which is the first step required in the TERIS scheme developed for the current study (described below).

The details of the procedures applied in Step 2 are presented in Figure 2. We searched the TERIS database for each medication in the verification list, and if the medication was present, we classified it according to the newly developed “TERIS scheme” (described

below). If the medication was absent from the TERIS database, we classified it based on our "expert consensus". The expert consensus was the opinion of two experts in teratogenicity and reproductive risk (B.M. and E.F.), who independently—and blinded from each other's opinions and results—classified each medication into either "to be included in Final List 1; teratogenic medications", "to be included in Final List 2; potentially teratogenic medications", or "to be included in neither list". The experts used all available published reports and resources to develop their ratings. For the inclusion into List 1, the experts used the criteria for proof of human teratogenicity proposed by Shepard.(Shepard, 1994) For a medication to be included into List 2, the experts used three stepwise conditions that the potential teratogen has to fully satisfy. Firstly, the experts verified that the medication did not meet Shepard's criteria (if it meets the criteria send it back to List 1, if no: proceed to second condition). Secondly, they examined if enough evidence of absence of teratogenic risk in humans already exists (if yes: to not include in neither list, if no: proceed to third condition). Thirdly, they examined if there is 1 human study or sufficient animal data that shows evidence of teratogenic risk (if yes: to include the medication in List 2, if no: to not include in neither list). The experts' opinions were collected by a third author (S.E.) and a consensus meeting was conducted to resolve any conflicting decisions.

The newly developed TERIS scheme is presented in Figure 3. In this scheme, we used the ratings available in the database to classify each medication in our Final lists. First, we looked at the quality and quantity of the data on which the risk rating was based. If it was "none", "very limited", or "limited", the medication was classified by the experts with our expert consensus procedure, as described above. If the rating was "fair", "good", or "excellent", we looked at the magnitude of the teratogenic risk. If it was "undetermined", then the medication was classified by the experts with our expert consensus procedure, as described above. If it was "none" or "unlikely", then it was rated as "to be included in neither lists"; if it was "minimal", then it was rated as "to be included in Final List 2: potentially teratogenic medications"; and if it was "small", "moderate", or "high", it was rated as "to be included in Final List 1: teratogenic medications". Whenever there was an intermediate rating by TERIS (e.g., quality and quantity of the data limited to fair), we used the highest rating (e.g., fair) to include as many medications as possible.

Statistical analysis

We tallied the number of medications included in each step with our classification procedure. We calculated the number and percentage of observed agreements between the two experts in teratogenicity. We also calculated the kappa value, with its 95% confidence interval (CI), and the weighted kappa for the agreement between the two experts. Measures of agreement were calculated with GraphPad Prism 2015 (GraphPad Software Inc. 2015, La Jolla, CA, USA).

Ethical approval

Because the study was conducted using online resources and medical references, with no human or animal involvement, no institutional review board approval was required.

RESULTS

In Step 1, 57 medications were included in each provisional list, so a total of 114 medications were verified for their exactness and completeness by the teratology expert. After verification, the classification of 57 medications was confirmed and they were included in the Final lists (43 in List 1: teratogenic medications and 14 in List 2: potentially teratogenic medications), whereas 57 medications required further verification and were entered onto the verification list. Fifty-two medications were identified by consulting other references, (Brent, 2004; Buonocore and others, 2010; Goodwin, 2010; Kalter, 2010; Koren and others, 1998; Malm and others, 2004; Polifka and Friedman, 2002; Porter and others, 2006; Queenan and others, 2010; Seyberth and others, 2011; Stevenson, 2006) together with 26 medications from Briggs et al. (2011) updates, so a total of 135 medications were entered onto the verification list, and then submitted to Step 2.

In Step 2, the TERIS scheme classified 23 medications (14 medications onto List 1: teratogenic medications; two medications onto List 2: potentially teratogenic medications; and seven medications were included on neither list) and 112 medications required classification by expert consensus, either because they did not appear in the TERIS database or when the quality and quantity of data was limited or the magnitude of teratogenic risk was undetermined (see Figure.3 TERIS scheme). From those 112 medications, 34 were classified onto List 1: teratogenic medications, 65 onto List 2: potentially teratogenic medications; and 13 medications were included on neither list. The

two experts agreed on the classification of 88 medications (78.57%) and the 24 medications upon which they differed were resolved by consensus (6 onto List 1: teratogenic medications, 13 onto List 2: potentially teratogenic medications; and five medications were included on neither list). To reach a consensus, evidence on each medication was reviewed by the experts and a third author (S.E.) in a closed meeting, and discussed until a common decision was reached. The strength of the agreement was considered "good", with kappa = 0.63 (95% CI: 0.50, 0.76) and weighted kappa = 0.65.

At the end of the two-step classification process, we had identified 91 teratogenic medications (List 1), presented in Table 1, and 81 potentially teratogenic medications (List 2), presented in Table 2.

DISCUSSION

Principal findings

In this report, we have presented a novel stepwise procedure for the classification of proven and potential teratogens, to be used by researchers in the fields of teratology, perinatology, perinatal epidemiology, and reproductive risk. The procedure utilizes existing reliable resources to obtain lists of medications with sufficient evidence of a teratogenic risk in the first trimester (referred to as "teratogenic medications"), and medications with a potential teratogenic risk in the first trimester (referred to as "potentially teratogenic medications"). Unlike previously published reports (Brent, 2004; Buonocore and others, 2010; Goodwin, 2010; Kalter, 2010; Koren and others, 1998; Malm and others, 2004; Polifka and Friedman, 2002; Porter and others, 2006; Queenan and others, 2010; Seyberth and others, 2011; Stevenson, 2006), we identified a substantial list of teratogenic medications, including 91 medications, and also an extensive list of potentially teratogenic medications, including 81 medications.

Comparison with other studies

The teratogenic medication lists available in the literature show significant discrepancies and have several drawbacks.(Buonocore and others, 2010; Goodwin, 2010; Kalter, 2010; Koren and others, 1998; Malm and others, 2004; Polifka and Friedman, 2002; Porter and others, 2006; Queenan and others, 2010; Seyberth and others, 2011; Stevenson, 2006;

Webster and Freeman, 2003) The problems associated with drawing up lists of teratogenic medications have been described at length in earlier reports.(Obican and Scialli, 2011; Scialli, 1997) The major problems lie in the imprecision and variability of the term "teratogen" and the errors that can arise when lists are used in counseling and clinical practice, not to mention the panic and anxiety they arouse in patients.(Obican and Scialli, 2011; Scialli, 1997) Nevertheless, such lists indeed have a significant importance in epidemiologic and clinical research. However, incomplete or inaccurate lists raise a major threat to the validity of research. The teratogen lists provided in earlier reports lack a systematic procedure for the classification of medications, even with the availability of appropriate references and peer-reviewed citations.(Andrade and others, 2006; Banhidy and others, 2005; Buonocore and others, 2010; Goodwin, 2010; Kalter, 2010; Malm and others, 2004; Porter and others, 2006; Seyberth and others, 2011; Stevenson, 2006; van Gelder and others, 2014b) The medication lists provided in the present report, constructed with the systematic procedure developed here, have several key advantages: 1) they come from a clear and systematic procedure; 2) they are easily updatable; 3) they provide a thorough list of potentially teratogenic medications, which is unprecedented.

The lists presented here are intended to be used for research. From the medications in List 1 (i.e., teratogenic medications), some may still be indicated during pregnancy to control a specific maternal condition (e.g., carbamazepine is indicated for seizure control in pregnant epileptic women, or lithium might have to be continued, including in the first trimester, by women suffering bipolar disorder and at risk of decompensation). Other List 1 medications might be contraindicated during pregnancy, but may have different magnitudes of risk. For example, maternal exposure to mycophenolate or isotretinoin during the first trimester is associated with a high risk of congenital malformations, whereas methimazole—also classified in List 1 here—is associated with a smaller increase in the overall risk of congenital malformations. Moreover, high risk can refer to the number of the exposed infants that become affected or to the severity of the congenital malformation itself. Our lists are intended for use in research and not in clinical practice. Several sources of accurate, free, evidence-based clinical counseling to healthcare professionals and patients are available through the Teratology Information Services, prominently the MotherToBaby (http://mothertobaby.org/) and ENTIS (http://www.entis-org.eu/) networks.

TERIS experts evaluate the teratogenic potential of a given medication. Their assessment is based on an in-depth analysis of the relevant published peer-reviewed studies and references, with an emphasis on human studies.(Adam and others, 2011) Their final ratings are obtained by consensus, after independent opinions are collected from experts in clinical teratology, birth defect epidemiology, experimental teratology, and related disciplines.(Adam and others, 2011) We noted that many drugs carry an "undetermined" risk rating in the TERIS database. These TERIS ratings reflect the absence of human data and the fact that animal studies might poorly predict the effects in humans.(Adam and others, 2011) Although animal data can provide useful information, they have some significant limitations, and certain requirements must be met to validly extrapolate animal results to human pregnancies.(Mazer-Amirshahi and others, 2014) In general, it is now agreed that we need all the data available on a medication, regardless of the source, to make a complete teratogenic risk assessment.(Scialli, 1997) Yet, the quality of the data could make us still unable to determine if a drug has the potential to be teratogenic in humans.

Strengths and limitations

The procedure we developed here is a systematic and updatable one, with objective components in most of its processes. The primary aim of this procedure is to provide lists of medications that can be used in research. However, the lists provided have potential utility in other areas. First, the list of potentially teratogenic medications (List 2) can effectively guide future research into medications that require further investigation in animal models. Second, the lists provide an encyclopedia of medications that require high-priority postmarketing surveillance.

Congenital malformations can arise when maternal exposure occurs above a threshold dose and at a critical time for the development of a specific fetal organ or system.(Ferreira and others, 2013) Because the majority of organs and systems develop in the first trimester—with the exception of the central nervous system—this is considered the period of highest fetal risk.(Banhidy and others, 2005) To maintain the consistency of the results, we considered the first trimester as the period of major interest in our classifications. Further research to develop lists of medications causing developmental damage in the second and third trimesters is warranted.

Antineoplastics include some of the most potent human and animal teratogens (e.g. methotrexate).(Cardonick and Iacobucci, 2004; Selig and others, 2012) Due to their targeted effects on vital cellular functions, the experts' decisions tended to include them in List 1, whenever their opinion was needed for their classification. Different medications in the same list might have different magnitudes of teratogenic risk, ranging from low to high. The lack of complete evidence about all the medications on the lists precludes any additional valid subclassification. Therefore, future research into this topic is highly recommended. The objectivity of the classification may have been compromised in instances in which expert opinion was required. However, we minimized this by blinding the reports of the experts and with the consensus process. The agreement between the experts' opinions in this study was good.

Reprotox and Shepard's Catalog of Teratogenic Agents are well-established teratogen information resources that we thought of including in the 2-Step procedure.(REPROTOX®; Shepard, 2010) However, neither Reprotox nor Shepard's Catalog of Teratogenic Agents have risk ratings or classifiable index as Briggs et al. reference book or the TERIS database. Therefore, in order to make the search and classification process systematic and reproducible, we chose to use them only when the experts classified the medications on their own and during their consensus meeting. We used the pregnancy recommendations given by Briggs et al. in our procedure (Step 1), but some potentially unsafe medications might have been included under the pregnancy recommendation "compatible-maternal benefit >> embryo-fetal risk" and were consequently not verified by our expert. However, Step 1 also included screening other references, in which such medications were cited. Because the literature is rapidly expanding, there might still be medications that were missed or of which we were unaware at the time we finalized the current report. When the data preparation of this report was finalized, the 9th edition of Briggs et al. was the latest available, and the 10th edition was published while the report was written. However, we updated our search in Briggs et al. to October 2013 and our search of TERIS to October 2014, with plans for future work based on the new edition of Briggs et al. (2015).(Briggs and others, 2015) We may have also overlooked some medications marketed outside North America in this study.

Implications for research

In most developed countries, over 90% of pregnant women use at least one medication—prescription medication, over-the-counter medication, or other supplement—during their pregnancy.(Daw and others, 2012; Ehrenstein and others, 2010) Pregnant women rarely participate in randomized controlled trials and evidence arising from observational studies has become central to the risk assessment of medications during pregnancy. However, observational research is characterized by various threats to internal validity, most importantly bias, including confounding. Therefore, confounding by indication and other potential confounders such as maternal age, race, smoking, alcohol consumption, chronic morbidities, other medication use, and obstetric history must be taken into consideration when analyzing the data.

In observational studies, the maternal exposure to potential and known teratogens is a constant concern for researchers. Indeed, the inability to accurately control for maternal teratogenic exposures can markedly threaten the validity of the study, causing a false association to become significant or masking a true one. The lists of medications presented in this report can be used in numerous ways in perinatal and reproductive epidemiologic research (e.g., exclusion of mothers exposed to them or by using various statistical adjustment techniques).

Summary

This report describes a systematic and updatable procedure for the classification of medications proven and potentially teratogenic when used during the first trimester of pregnancy. This procedure has identified a large number of medications that were not reported in similar previous reports. These exhaustive lists of proven and potential teratogens will be of a substantial value in teratology research and related fields.

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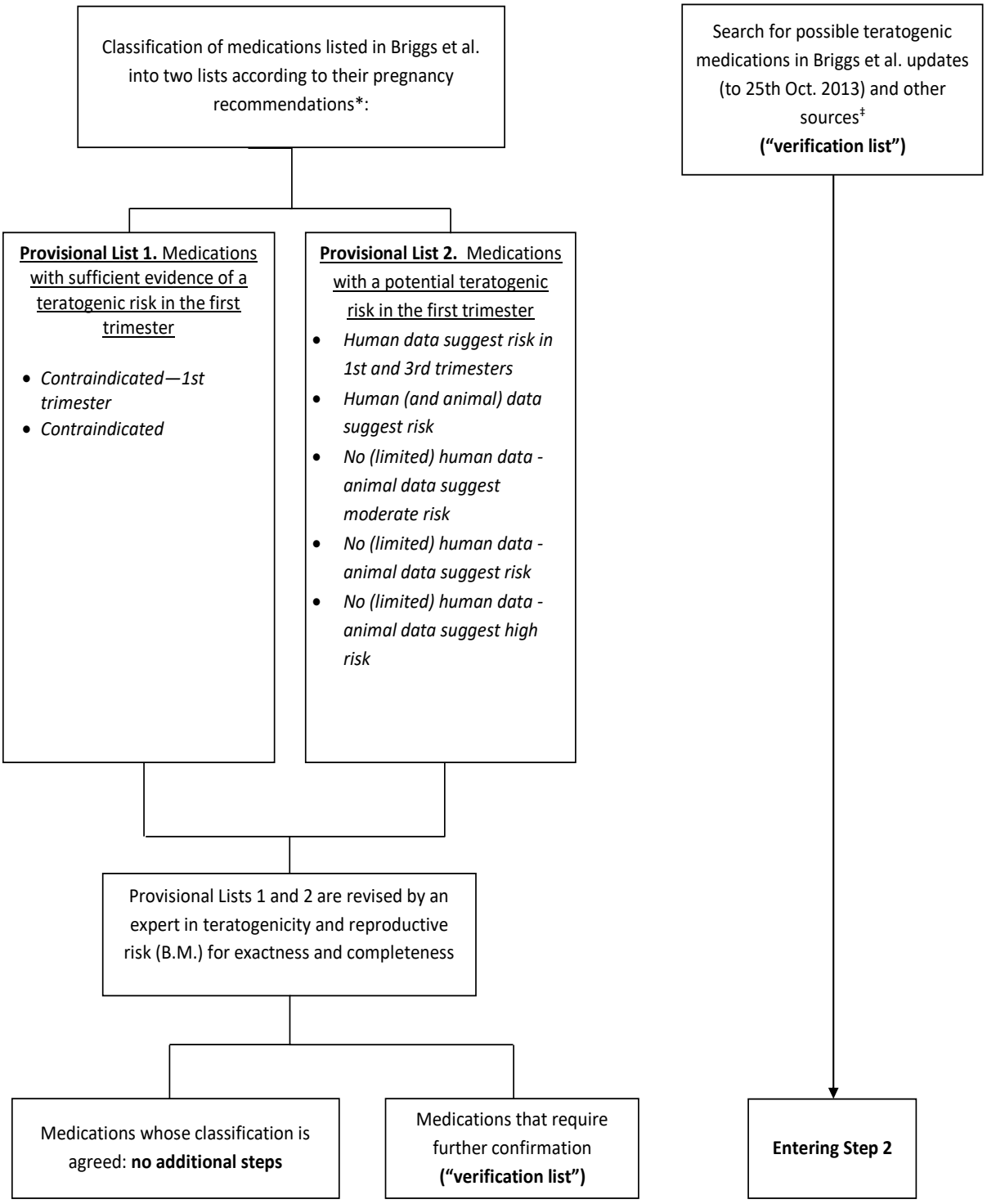
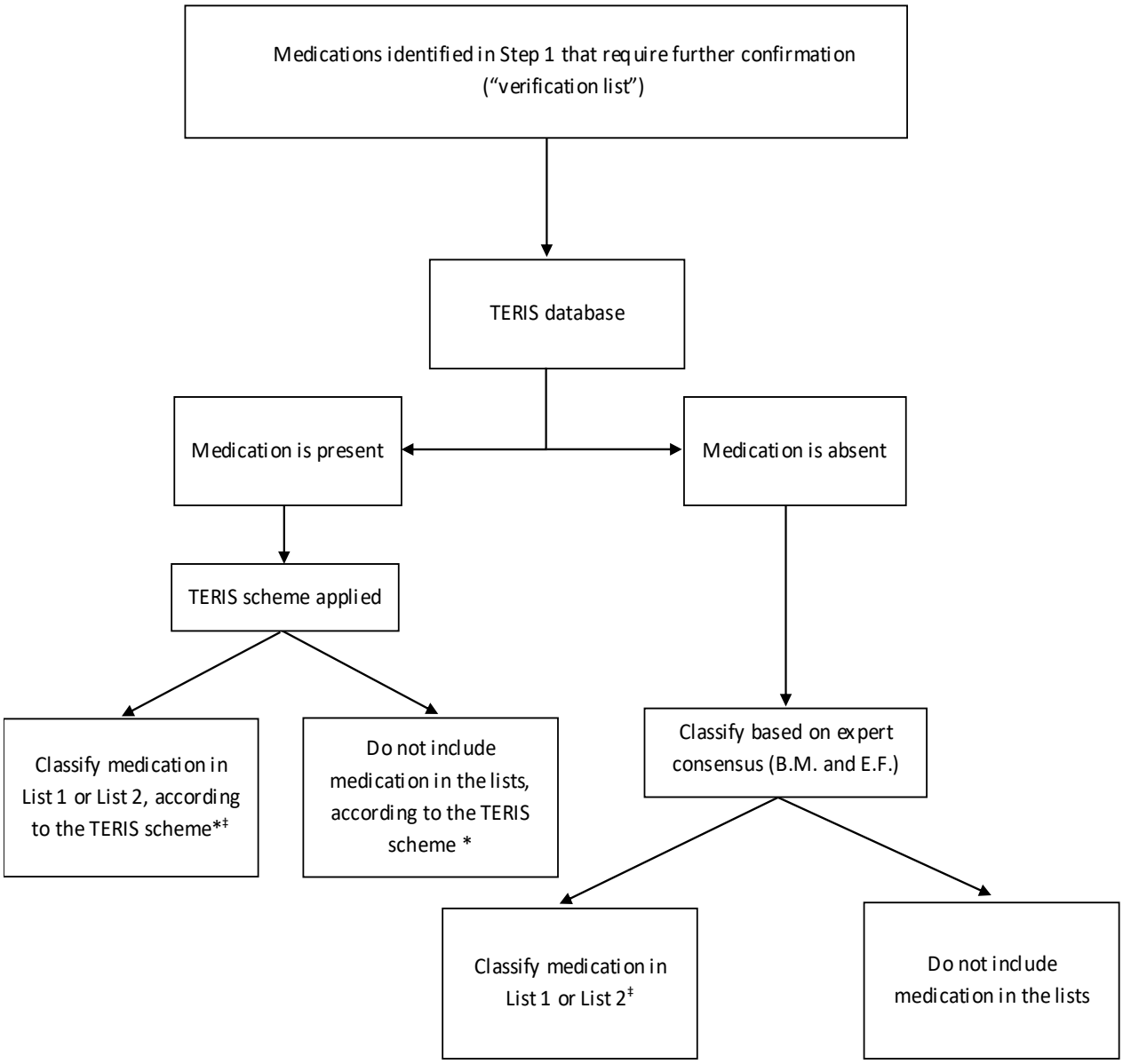
Figure 1. Flow diagram of the classification of medications with a teratogenic risk or a potential teratogenic risk in the first trimester: **Step 1**



*See Appendix 1 for complete definitions of pregnancy recommendations.

‡ Brent, 2004; Buonocore and others, 2010; Goodwin, 2010; Kalter, 2010; Koren and others, 1998; Malm and others, 2004; Polifka and Friedman, 2002; Porter and others, 2006; Queenan and others, 2010; Seyberth and others, 2011; Stevenson, 2006

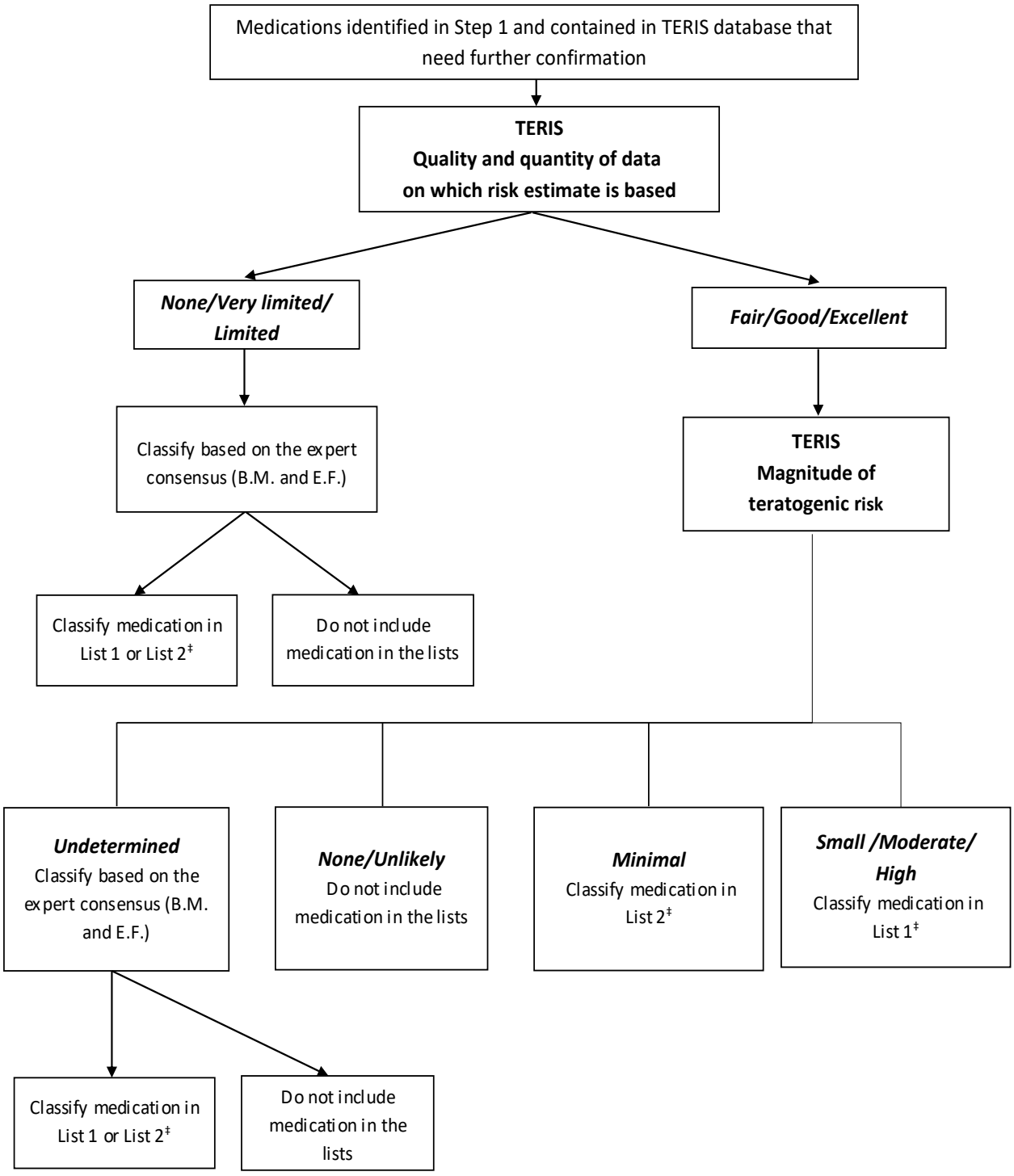
Figure 2. Flow diagram of the classification of medications with a teratogenic risk or a potential teratogenic risk in the first trimester: **Step 2**



* See Figure 3 for details of the classification procedure of drugs based on the TERIS scheme.

‡ List 1: Medications with sufficient evidence of a teratogenic risk in the first trimester; List 2: Medications with a potential teratogenic risk in the first trimester.

Figure 3. Classification scheme for medications through TERIS



‡ List 1: medications with sufficient evidence of a teratogenic risk in the first trimester; List 2: medications with a potential teratogenic risk in the first trimester.

Table 1. Medications with sufficient evidence of a teratogenic risk in the first trimester*

1. acenocoumarol
2. acitretin
3. alitretinoin
4. amethopterin
5. amsacrine
6. axitinib
7. bishydroxycoumarin
8. bleomycin
9. brentuximab
10. busulfan
11. capecitabine
12. carbamazepine
13. carbimazole
14. carboplatin
15. carmustine
16. chlorambucil
17. cisplatin
18. cladribine
19. colchicine
20. crizotinib
21. cyclophosphamide
22. cytarabine
23. dacarbazine
24. dactinomycin
25. danazol
26. daunorubicin
27. diethylstilbestrol
28. docetaxel
29. doxorubicin
30. epirubicin
31. estramustine
32. etoposide
33. etretinate
34. exemestane
35. flucytosine
36. fludarabine

37. fluorouracil
38. fluoxymesterone
39. formestane
40. gemcitabine
41. idarubicin
42. ifosfamide
43. imatinib
44. iodine-125 / iodine-123
45. iodine-131
46. isotretinoine
47. l-asparaginase
48. leflunomide (animal data)
49. lenalidomide
50. lithium
51. lomustine
52. mechlorethamine
53. medroxyprogesterone
54. melphalan
55. mephenytoin
56. mephobarbital
57. methandrostenolone
58. methimazole
59. methotrexate
60. methyltestosterone
61. misoprostol
62. mitomycin
63. mitoxantrone
64. mycophenolate mofetil (MMF)
65. nicoumalone
66. oxaliplatin
67. paclitaxel
68. paramethadione
69. pemetrexed
70. penicillamine
71. phenobarbital
72. phenytoin
73. primidone
74. procarbazine
75. ribavirin

76. tamoxifene
77. temozolomide
78. teniposide
79. testosterone
80. thalidomide
81. thioguanine
82. thiotepa
83. tretinoin (systemic)
84. trimethadione
85. trimethoprim
86. valproic acid/divalproex
87. vinblastine
88. vincristine
89. vindesine
90. vinorelbine
91. warfarin

* This list is intended to be used by researchers in the context of bias control in epidemiological or clinical studies. It is not meant for use in clinical settings by health-care providers or patients, because this classification does not take into account the clinical context of the exposure (route of administration, dose, time of exposure, etc.) and does not provide an estimate of the magnitude of the teratogenic risk during pregnancy.

Whenever there was an intermediate rating by TERIS (e.g., quality and quantity of the data limited to fair), we used the highest rating (e.g., fair) to include as many medications as possible.

Table 2. Medications with a potential teratogenic risk in the first trimester*

1. abiraterone
2. acetylsalicylic acid
3. ambrisentan
4. amiodarone
5. azathioprine
6. benazepril
7. busereline
8. candesartan
9. captopril
10. carboprost
11. carglumic acid
12. cetrorelix
13. cilazapril
14. clobazam
15. clomiphene
16. clomipramine
17. dabigatran
18. dalfampridine
19. degarelix
20. denosumab
21. dexmedetomidine
22. DHEA (dehydroepiandrosterone)/ prasterone
23. dinoprostone
24. dronedarone
25. eculizumab
26. enalapril
27. eprosartan
28. ergotamine
29. etomidate
30. ezogabine
31. fingolimod
32. fluconazole: high dose or chronic use
33. follitropine alpha
34. follitropine beta
35. fosinopril
36. gliclazide
37. goserelin

38. haloperidol
39. hydroxyurea
40. indomethacin
41. iodixanol
42. ipilimumab
43. irbesartan
44. lamotrigine
45. letrozole
46. levetiracetam
47. lisinopril
48. losartan
49. loxapine
50. lutropin alfa
51. medroxyprogesterone
52. megestrol
53. miglustat
54. nandrolone
55. nateglinide
56. norethandrolone
57. olmesartan
58. oxcarbazepine
59. oxymetholone
60. paroxetine
61. perindopril
62. phenelzine
63. phensuximide
64. pimozide
65. pioglitazone
66. quinapril
67. quinine
68. raloxifene
69. ramipril
70. repaglinide
71. riluzole
72. rivaroxaban
73. rosiglitazone
74. stanozolol
75. telmisartan
76. tesamorelin
77. topiramate

78. ulipristal
79. urofollitropine (FSH)
80. valsartan
81. vigabatrin

* This list is intended for use by researchers in the context of bias control in epidemiological or clinical studies. It is not meant for use in clinical settings by health-care providers or patients, because this classification does not take into account the clinical context of the exposure (route of administration, dose, time of exposure, etc.) and does not provide an estimate of the magnitude of the teratogenic risk during pregnancy.

Whenever there was an intermediate rating by TERIS (e.g., quality and quantity of the data limited to fair), we used the highest rating (e.g., fair) to include as many medications as possible.

Appendix 1: Definitions of Pregnancy Recommendations in Briggs et al. 2011, *Drugs in Pregnancy and Lactation: a Reference Guide to Fetal and Neonatal Risk* (9th ed.)

Provisional List 1. Medications with sufficient human evidence of a teratogenic risk in the first trimester or antineoplastics

- ***Contraindicated - 1st Trimester***

Human exposures in the 1st trimester, either to the drug itself or to drugs in the same class or with similar mechanisms of action, have been associated with developmental toxicity (growth restriction, structural anomalies, functional/behavioral deficits, or death). The drug should not be used in the 1st trimester.

- ***Contraindicated***

Human exposures at any time in pregnancy, either to the drug itself or to drugs in the same class or with similar mechanisms of action, have been associated with developmental toxicity (growth restriction, structural anomalies, functional/behavioral deficits, or death). Animal reproduction data, if available, confirm the risk. The drug should not be used in pregnancy.

Provisional List 2. Medications with a potential teratogenic risk in the first trimester based on human and/or animal data

- ***Human Data Suggest Risk in 1st and 3rd Trimesters***

Evidence (for the drug or similar drugs) suggests that there may be an embryo-fetal risk for developmental toxicity (growth restriction, structural anomalies, functional/behavioral deficits, or death) in the 1st and 3rd trimesters but not in the 2nd trimester. The human pregnancy data outweigh any animal reproduction data.

- ***Human (and Animal) Data Suggest Risk***

The human data for the drug or drugs in the same class or with the same mechanism of action, and animal reproduction data if available, suggest there may be a risk for developmental toxicity (growth restriction, structural anomalies, functional/behavioral deficits, or death) throughout pregnancy. Usually, pregnancy exposure should be avoided, but the risk may be acceptable if the maternal condition requires the drug.

- ***No (Limited) Human Data - Animal Data Suggest Moderate Risk***

Either there is no human pregnancy experience or the few pregnancy exposures have not been associated with developmental toxicity (growth restriction, structural anomalies, functional/behavioral deficits, or death). The drug causes developmental toxicity (at doses that did not cause maternal toxicity) in one animal species at doses ≤10 times the human dose based on body surface area (BSA) or AUC.

- ***No (Limited) Human Data - Animal Data Suggest Risk***

Either there is no human pregnancy experience or the few pregnancy exposures have not been associated with developmental toxicity (growth restriction, structural anomalies, functional/behavioral deficits, or death). The drug causes developmental toxicity (at doses that did not cause maternal toxicity) in two animal species at doses ≤10 times the human dose based on body surface area (BSA) or AUC.

- ***No (Limited) Human Data - Animal Data Suggest High Risk***

Either there is no human pregnancy experience or the few pregnancy exposures have not been associated with developmental toxicity (growth restriction, structural anomalies, functional/behavioral deficits, or death). The drug causes developmental toxicity (at doses that did not cause maternal toxicity) in three or more animal species at doses ≤10 times the human dose based on body surface area (BSA) or AUC.

Definitions excluded from selection

- ***Compatible***

The human pregnancy experience, either for the drug itself or drugs in the same class or with similar mechanisms of action, is adequate to demonstrate that the embryo-fetal risk is very low or nonexistent. Animal reproduction data are not relevant.

- ***No (Limited) Human Data - Probably Compatible***

There may or may not be human pregnancy experience, but the characteristics of the drug suggest that it does not represent a significant risk to the embryo-fetus. For example, other drugs in the same class or with similar mechanisms are compatible or the drug does not obtain significant systemic concentrations. Any animal reproduction data are not relevant.

- ***Compatible - Maternal Benefit >> Embryo-Fetal Risk***

There may or may not be human pregnancy experience, but the potential maternal benefit far outweighs the known or unknown embryo-fetal risk. Animal reproduction data are not relevant.

- ***Human Data Suggest Low Risk***

There is limited human pregnancy experience, either for the drug itself or drugs in the same class or with similar mechanisms of action, including the 1st trimester, suggesting that the drug does not represent a significant risk of developmental toxicity (growth restriction, structural anomalies, functional/behavioral deficits, or death) at any time in pregnancy. The limited human pregnancy data outweighs any animal reproduction data.

- ***No (Limited) Human Data - Animal Data Suggest Low Risk***

Either there is no human pregnancy experience or the few pregnancy exposures have not been associated with developmental toxicity (growth restriction, structural anomalies, functional/behavioral deficits, or death). The drug does not cause developmental toxicity (at doses that did not cause maternal toxicity) in all animal species studied at doses ≤10 times the human dose based on body surface area (BSA) or AUC.

- ***Contraindicated - 2nd and 3rd Trimesters***

Human exposures in the 2nd and 3rd trimesters, either to the drug itself or to drugs in the same class or with similar mechanisms of action, have been associated with developmental

toxicity (growth restriction, structural anomalies, functional/behavior deficits, or death). The drug should not be used in the 2nd and 3rd trimesters.

- ***No (Limited) Human Data - No Relevant Animal Data***

There is no human pregnancy data or relevant data in animals, or the human pregnancy experience, that may or may not include the 1st trimester, is limited. The risk in pregnancy cannot be assessed.

- ***Human Data Suggest Risk in 2nd and 3rd Trimesters***

Evidence (for the drug or similar drugs) suggests that there may be a fetal risk for developmental toxicity (growth restriction, structural anomalies, functional/behavioral deficits, or death) in the 2nd and 3rd trimesters but not in the 1st trimester. The human pregnancy data outweigh any animal reproduction data.

- ***Human Data Suggest Risk in 3rd Trimester***

Evidence (for the drug or similar drugs) suggests that there may be a fetal risk for developmental toxicity (growth restriction, structural anomalies, functional/behavioral deficits, or death) in the 3rd trimester, or close to delivery but not in the 1st or 2nd trimesters. The human pregnancy data outweigh any animal reproduction data.

5.5 Correspondence to *The Journal of Allergy and Clinical Immunology*

Asthma during pregnancy and congenital malformations: the challenging task of separating the medication effect from asthma itself

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This correspondence is included in the current thesis by the permission of the co-authors and editors.

Asthma during pregnancy and congenital malformations: the challenging task of separating the medication effect from asthma itself

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Declaration of funding: None.

To the Editor: We have read with interest the article by Garne et al.(1) which adds new information on the impact of asthma treatments during pregnancy on the prevalence of congenital malformations. The study concluded that the use of inhaled β_2-agonists (short and long-acting combined) is associated with an increased risk of cleft palate and gastroschisis, while inhaled corticosteroids (ICS) showed no increased risk for any of the examined malformations. We fear however that such statement could negatively affect the clinicians and mothers' confidence in short-acting β_2-agonists (SABA) – specifically salbutamol– which was the most frequently used β_2-agonist in this study. We believe that methodological limitations led to the observed results and should be carefully considered.

The first limitation in the study is the use of a reference group formed of asthmatic and non-asthmatic women. Including non-asthmatic women in the reference group could potentially overestimate the effect of the asthma medications. Asthma itself is associated with an increased risk of congenital malformations, and a recent meta-analysis reported a 30% increased risk of cleft lip among asthmatic as compared to non-asthmatic women.(2) In the study by Garne et al., 53% of asthmatic women treated with β_2-agonists (86% using salbutamol) had no controller medications and were likely having uncontrolled asthma. On the other hand, the ICS group is likely to include women who were appropriately controlled due to the beneficial effect of ICS. Due to this confounding by control level, an increased prevalence of malformations was found in the β_2-agonists group – corroborating results from previous case-control studies using similar exposure groups (3-5) – and not found among the ICS group (even showing protective effects in some instances). Moreover, the authors did not report the maternal characteristics of the women in the study (e.g. age, comorbidities, asthma exacerbations, hospitalizations for asthma and oral corticosteroids use), which prevented the assessment of the comparability of the exposure groups.

SABA (salbutamol in particular) have shown fetal safety in several well designed cohort and case-control studies. In fact, in the recent Swedish study(6) cited by the authors, the observed increased risk of cleft palate with bronchodilator use was the lowest for SABA and no increased risk of gastroschisis was observed. Disentangling the effect of the medication from the disease is a challenging task that has to be appropriately addressed in both the design and the analysis of the study. Most importantly, the use of a reference group

formed of women with asthma is recommended. Researchers have also proposed indirect ways to separate the medications' effects, including a comparison between different drugs that share similar indications,(6) and such studies were recently published in the literature.(7, 8)

Other limitations in the study include the lack of adjustment for important confounders such as socioeconomic status and asthma exacerbations, combining short and long acting β_2-agonists under one exposure category, and multiple comparisons. While we acknowledge the use of the case-malformed control design, the rationale behind its use is questionable since the exposure information was recorded prospectively. The use of healthy controls without any apparent pathology could have been more appropriate, especially in confirming signals from previous studies that used non-malformed controls.

Conflict of Interest: None

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CHAPTER 6: DISCUSSION

Discussion

6.1 General discussion

Considering the relatively high and increasing prevalence of asthma among pregnant women, coupled with the increasing use of different treatment regimens with incomplete evidence on their safety during pregnancy, we sought to examine the comparative safety of two of the most widely used treatment regimens for asthma during pregnancy. Through our endeavor, we tackled some intriguing questions we thought could add important knowledge in this field.

We first conducted a systematic review to summarize the published evidence on the impact of maternal use of SABA and LABA during pregnancy and different perinatal outcomes. We found few studies that reported significant increased risk of congenital malformations for women exposed to SABA and/or LABA and no increased risk was found for the other outcomes. Importantly, most of the retrieved studies suffered several methodologic limitations which we described in our systematic review. Moreover, the non-significant results from the studies should be interpreted with caution since a large percentage of the negative studies were underpowered to detect clinically significant effects. We made several recommendations on how to tackle these limitations and the possible future research plans to obtain precise estimates of the associated risks to rule on the SABA and LABA safety profiles.

We then compared the risk of major congenital malformations in pregnant asthmatic women treated with a combination of LABA-ICS and those treated with a higher dose of ICS monotherapy. Indeed, there has been no direct comparison of these treatment regimens before to guide physicians in whether it is safer for the newborn to increase the dose of ICS during pregnancy or to add a LABA to the current ICS dose used. Through analysing comparable treatment regimens and classifying the asthmatic women into two groups based on the asthma medications they used to control their symptoms, we were able to obtain relatively unbiased results while minimizing confounding by asthma severity. We found that the risk of major malformations did not differ when a combination therapy of LABA-ICS or a higher dose of ICS monotherapy was used in the first trimester of pregnancy

among moderate to severe asthmatic pregnant women (aOR: 1.0; 95% CI: 0.6–1.7 for the two subcohorts combined). We concluded that the findings support the fetal safety of LABA-ICS combination in the management of persistent asthma during pregnancy.

We also investigated the impact of different case ascertainment definitions for major congenital malformations on the estimated prevalence and the maternal asthma-major malformations association. We demonstrated through a series of analyses that the source of data and the classification method had a considerable impact on the prevalence of major malformations, but a small influence on the aORs. Adding ≥ 1 or 2 medical claim diagnoses to hospital-based diagnoses increased the prevalence of major malformations by 10.0% and 38.8%, respectively, for the TCMC method (a method that we developed for congenital malformations research) and by 13.7% and 50.4%, respectively, for the CCASS method (the national Canadian surveillance method). In terms of classification methods, the CCASS led to higher prevalence of major malformations than the TCMC, mainly because some minor malformations were classified as major malformations. We concluded that the case ascertainment definition based on ≥ 1 hospitalization diagnosis combined with the TCMC classification method is likely to be the most reliable, since it has the least chance of including misclassified and false positive cases.

Finally, we aimed at constructing a systematic procedure for the classification of proven and potential teratogenic medications during the first trimester of pregnancy, to be used for research. Given that the teratogenic medication lists available in the literature show significant discrepancies and have several drawbacks, the challenging task was establishing the procedure itself when no similar precedent approach or procedures has been published previously in the literature. We structured a procedure that is both systematic and updatable, with objective components in most of its processes. We identified a substantial list of teratogenic medications, including 91 medications, and an extensive list of potentially teratogenic medications, including 81 medications. The identified lists could carry a substantial value in teratology research and several other related fields.

6.2 Contribution of our results to the literature in the fields of maternal asthma, perinatal outcomes and teratogenicity

6.2.1 Asthma treatments and perinatal outcomes

Beta$_2$-agonists have a crucial role in asthma management during pregnancy.[5,14,21-30] Recent reports suggest that 40 to 70% of asthmatic women use SABA and 8 to 13% use LABA during pregnancy.[12,134] Several studies have examined the perinatal safety of SABA and LABA, with both negative and positive findings.[21-30] Systematic reviews on the topic are essential as they summarize the available body of knowledge for better evidence-based decision making. Published reviews on this topic did not capture the whole evidence from all published studies on the different clinically important perinatal outcomes.[2,129]. In our systematic review, we presented a critique for the published studies, in which we elaborated on their strengths and weaknesses and assessed their quality using the recommended Newcastle-Ottawa scale. We also performed a series of post-hoc power calculations to identify studies able to detect clinically significant effects.

From the publications that we have reviewed, we found evidence of increased risk of congenital malformations after maternal exposure to fenoterol (SABA) in one study[23] and LABA in another study[30], but we could not rule out the presence of residual confounding by indication. No increased risk was found for the other outcomes, except a decrease in birth weight centiles among salmeterol (LABA) users.[136] The largest body of evidence on the safe use during pregnancy was available for salbutamol (SABA). The different methodologic limitations prevented drawing precise conclusions on the safety profiles of all SABA and LABA, especially with the presence of confounding by asthma severity and a plentiful of underpowered negative studies. Therefore, more research is warranted on the use of SABA and LABA during pregnancy to rule on their safety profile.

Women suffering from persistent asthma have to be treated with the appropriate medications to control their asthma symptoms including exacerbations.[5,14] Both ICS monotherapy and LABA-ICS combinations are widely used during pregnancy, with an increasing number of users over time.[269] The guidelines of asthma management during pregnancy recommends both treatment regimens and the physicians generally decide based on individual experiences and patients' preferences and needs, among many other

factors.[14,96] Yet, the comparative safety of those two treatment regimens are not well described.

We conducted the first comparative safety study that answers the specific question of comparing the risk of major congenital malformations between LABA-ICS combination versus ICS monotherapy in higher doses among pregnant women with moderate to severe asthma. The study showed that the risk of major malformations did not differ when a combination therapy of LABA plus ICS or a higher dose of ICS monotherapy was used in the first trimester of pregnancy. The results were consistent in both subcohorts of moderately and severely asthmatic pregnant women (aOR: 1.1; 95% CI: 0.6–1.9 and aOR: 1.2; 95% CI: 0.5–2.7 respectively). We minimized confounding by indication by performing the primary analysis within subcohorts of women with similar levels of asthma severity and by adjusting for baseline severity markers. These reassuring results provide scientific evidence to help physicians and mothers make evidence-based treatment decisions during pregnancy.

6.2.2 Case ascertainment definitions of major malformations

In this methodologic study, we aimed to compare the prevalence of major malformations using different case ascertainment definitions that vary by the source of data and the classification method. We also evaluated the impact of these definitions on the association between maternal asthma and major malformations. Previous reports have examined the validity of the congenital malformation diagnoses recorded in RAMQ and MED-ECHO,[60,61] but none has examined each separately, especially the impact of using the medical claims diagnoses – in RAMQ database – as an additional source of data with the hospitalizations database. Using six different case ascertainment definitions, we showed that medical claims can strongly affect the prevalence of major malformations estimates, corroborating the results from similar studies using Canadian provincial databases. [55,248] We tested in this study the suitability of the new classification method (i.e. the TCMC method), developed previously by our research team, in congenital malformations research. We compared it to an established classification method used by the national surveillance system in Canada (i.e. the CCASS method). The results provided significant assurance to the suitability of the TCMC method, showing superiority for research purposes as compared to

the CCASS method which better suits the surveillance needs. The study also showed that the case ascertainment definitions had only a small influence on the aORs estimating the association between asthma and congenital malformations. The study results and our recommendations could assist in guiding future research on congenital malformations and the comparative effectiveness and safety of drug therapies during pregnancy.

6.2.3 Medications with proven and potential teratogenic risk

Pregnant women rarely participate in randomized controlled trials and evidence arising from observational studies has become central to the risk assessment of medications during pregnancy. Yet in observational studies, the maternal exposure to potential and known teratogens is a constant concern for researchers. Several well-known teratogen information databases and references are currently available, including Briggs et al. reference book, TERIS, Reprotox and Shepard's Catalog of Teratogenic Agents, each provide either complete or partial evidence for the teratogenicity of medications.

We searched the published literature for proven and potential teratogenic medications lists. We observed significant discrepancies between the lists of medications that should be considered teratogenic, and additional imprecisions were found when categories are used (e.g., moderate- vs high-risk teratogens).[62-73,233] Some researchers prefer developing their own lists which they regularly update and use to conduct their research. The main limitation in such case is that the selection process becomes primarily subjective and never described in details, which prevents reproducibility and adoption by other researchers. We were unable to locate neither a systematic procedure nor an easy-to-update lists of proven and potential teratogens.

In our study, we provided lists of medications which are up-to-date, until the most recent evidence at the publication time, but more importantly we developed a systematic procedure that can be used to update the lists whenever needed. The lists presented can be used in numerous ways in perinatal and reproductive epidemiologic research (e.g., exclusion of mothers exposed to them or by using various statistical techniques). We recommend that other researchers adopt our lists in their future research. The lists also have potential value in other areas. For example, the list of potentially teratogenic medications

can effectively guide future research into medications that require further investigation in animal models, and medications that require high-priority postmarketing surveillance.

6.3 Strengths, limitations and internal and external validity

6.3.1 Systematic review on beta$_2$-agonists and perinatal outcomes

The presented systematic review has some key strengths. Prior to commencing our search, a systematic review protocol was formed, registered and published in PROSPERO, the International prospective register of systematic reviews. We searched six different databases for original articles, besides the reference lists from retrieved articles, which allowed us to include the most relevant studies that provided information on the perinatal outcomes we examined. We covered in this review most of the essential outcomes that best represent the fetal development (major and any malformations, SGA, mean and low birth weight) and the newborn prematurity (gestational age and preterm delivery). We used the validated and recommended NOS-scale for the quality assessment of the studies. We used well-known reporting guidelines, the PRISMA statement, to ensure effective reporting of our results. In addition, we performed a post-hoc power calculation for each study included in the systematic review to identify studies that had adequate power to detect clinically significant effects. The performed power calculations in our review were original and a first, since previous systematic reviews on beta$_2$-agonists lacked this important component. Compared to previously published reviews on the effect of beta$_2$-agonist use during pregnancy, the spectrum of perinatal outcomes investigated in the current review was larger.

The review had some limitations. The main limitation was the fact that we could not pool the different study results into a single estimate for each outcome due to major methodological differences between the studies. Another limitation is that the review included only studies published in English language. Moreover, we excluded studies that did not have comparison groups (i.e. case-reports and case-series). However, those studies have several inherent limitations that could have affected the quality of the systematic review.

6.3.2 Articles on LABA-ICS combination versus ICS monotherapy and case ascertainment definitions of major malformations

6.3.2.1 Databases

Among the major strengths of the two conducted studies is the use of the Quebec Asthma and Pregnancy Database. This database is considered one of the largest administrative-linked pregnancy databases in Canada, spanning over 20 years. The database includes 583,071 pregnancies, representing about 35% of all births in the province in this period of time.[260] A remarkable advantage in the database is that it includes all pregnancies from asthmatic women in Quebec over a 20 years period. Indeed, the Quebec Asthma and Pregnancy Database is one of the largest worldwide in terms of the number of pregnancies from women with asthma (i.e. 36,587 pregnancies). The database was constructed through the linkage of two large health administrative databases from Quebec that have their unique strengths in research applications. Using this prospectively gathered and interlinked databases to identify the exposures and outcomes provided several advantages (as summarized below) over other methods of data collection such as self-reported questionnaires or maternal interviews.[236,270]

Different studies have shown that most patients have difficulties reporting the details of their medication use, for example the time, the doses, and the quantity used.[271-274] In the article on LABA-ICS combination versus ICS monotherapy, data on filled prescriptions, which were used to assess the women's exposure to asthma medications during pregnancy, were prospectively collected independently of the outcome, avoiding any recall bias, which is common in reproductive research.[236,270] Moreover, using the health administrative databases allowed us to capture the history of the medication use over long periods (three months before and during the pregnancy period) for a large number of patients. Computerized health databases also provide the chance to study a sizable number of patients with a reasonable budget and time-frame.

The prescription data recorded in the RAMQ database have been formally evaluated and found to be accurate and valid (83% correct identification of the patients and drugs dispensed from the prescriptions).[262] The validity of the diagnoses of asthma recorded in the RAMQ and MED-ECHO databases has been formally evaluated and the data were shown to have a PPV of 75% and a PNV of 96% for asthma diagnoses.[261] In the article on

LABA-ICS combination versus ICS monotherapy, the use of two large administrative databases allowed us to access a large number of pregnancies in asthmatic women, from which we could establish our subcohorts and measure several potentially important confounders. Using a large database allowed us to perform our statistical analysis for the two subcohorts with reasonable statistical powers (a power of 80% to detect an OR of 1.9 and 2.4 in the two subcohorts), and allowed to perform a sensitivity analysis – using the two subcohorts combined – with a higher statistical power (a power of 80% to detect an OR of 1.7).

Pregnancy variables recorded in the RAMQ and MED-ECHO databases have been formally evaluated and found to be highly valid.[261] From the variables that have been validated and were used to perform our analyses are maternal age, length of gestation, date of delivery, and date of last menstruation.[261] The validity of the variables was assessed by calculating Pearson correlation coefficient between the values obtained from the databases and patents' medical charts, and the correlations were found to be high for all variables ranging from 0.920 to 0.999.[261]

In the methodologic study, we used a validated definition of asthma. This operational definition was developed in Ontario health databases and previously validated. The validation study showed a sensitivity of 83.8% and a specificity of 76.5% as compared to patients' charts from primary care physicians' practices.[264] Our methodologic study is the first to examine the number of additional cases of major malformations identified in outpatient medical claims database in Quebec, and the first to compare the CCASS classification method used for national surveillance to the TCMC method designed specifically for perinatal research. In this study we tested and compared the use of several case ascertainment definitions, providing a recommendation on the suitability of using the TCMC and hospitalization diagnoses for ascertainment of major congenital malformations. This recommendation was followed in our article on LABA-ICS combination versus ICS monotherapy. The validity of the diagnoses of congenital malformations recorded in the RAMQ and MED-ECHO databases has been formally evaluated and the data were shown to have a PPV of 82% and a PNV of 88% as compared to data from the infants' medical charts.[61]

6.3.2.2 Study methodology

Our choice for the studies' design – being retrospective cohort studies – was suitable for our objectives as this design is highly efficient in terms of timeframe and cost-effectiveness. The choice of the reference group is a determining factor in the validity of the results obtained from observational studies. Using non-asthmatics as a reference group carries a potential risk to the study validity. As we previously exhibited in the literature review chapter, asthma itself – and asthma symptoms including exacerbations – have been shown to be associated with several adverse perinatal outcomes, including congenital malformations.[2,5,7-9] Consequently, confounding by indication (i.e. asthma itself) should be considered at the design stage of the study.

In the article on LABA-ICS combination versus ICS monotherapy, we presented the first study – to the best of our knowledge – that compared the risk of congenital malformations for different comparable treatment options for the management of moderate to severe asthma during pregnancy. Previous studies have compared women treated with LABA or ICS with either asthmatic women not exposed to the medication or nonasthmatic women, leading to overestimating the true effect of the treatments alone.[275] We also minimized confounding by indication through performing the primary analysis within subcohorts of women with similar levels of asthma severity and by adjusting for baseline severity markers.

6.3.2.3 Limitations of the studies

Random error

Random error (i.e. chance effect) is defined as the variability in the data and it represent the precision of the observed estimates.[236,276] Random error usually diminishes as sample size gets larger.[236,270] A small P-value and a narrow confidence interval are reassuring signs against chance effect.[270,276] In the article on LABA-ICS combination versus ICS monotherapy, we had a large cohort of pregnancies allowing for relatively adequate statistical power. We had a statistical power of 80% to detect an OR of 1.9 in the moderate asthma subcohort and an OR of 2.4 in the severe asthma subcohort, and associations smaller than that might not have been detected in our primary analysis.

However, the secondary analysis, which combined the two subcohorts, had a power of 80% to detect an OR of 1.7.

Systematic error

Systematic error (i.e. bias) mainly influences the internal validity of the study. Bias refers to any systematic process in the conduct of a study that leads to deviation from the truth and incorrect observed estimates.[236,270] It could result due to errors in the way the subjects were selected, errors in the measurement of variables, or any confounding factor that is not completely controlled for.[236,270] Generally, systematic bias can be classified into selection bias, information bias, and confounding bias.[236,270]

Selection bias refers to any error that arises in the process of selecting study subjects, and in a cohort study it is frequently related to losses to follow-up. [236,270] In the two cohort studies we conducted, we don't believe that we faced a situation in which this kind of bias could have strongly affected the validity of our results. The selection process was the same between the groups compared, but a potential loss to follow-up could have occurred if a woman become uninsured by the RAMQ during her pregnancy period. The direction and magnitude of this bias is unknown since we do not have data on the number of women who were lost to follow-up nor if it was differential or not between our compared groups. We suggest however that this bias did not strongly affect our results since we expect the number women in this group to be relatively small.

Other potential sorts of selection bias might be present. Our cohort is selected from a sample of pregnancies that completed 20 weeks of gestation and our assessed exposures (i.e. LABA and ICS use) were measured prior to the 20th week of gestation. In the first scenario (see directed acyclic graph [DAG] in Fig. 6.3.1), a possible selection bias could have occurred if early termination of pregnancies (a possible cause of LABA or ICS) was associated with major malformations in our study (the dashed arrow(s) in Fig. 6.3.1). If this association is present, then a non-causal pathway could have been opened that led to biased results. The bias will be differential only if one treatment regimen causes more early termination of pregnancies than the other regimen, and the deviation from the truth will depend on which regimen it is. No data is available on the early terminations' prevalence among LABA-ICS users versus ICS monotherapy users.

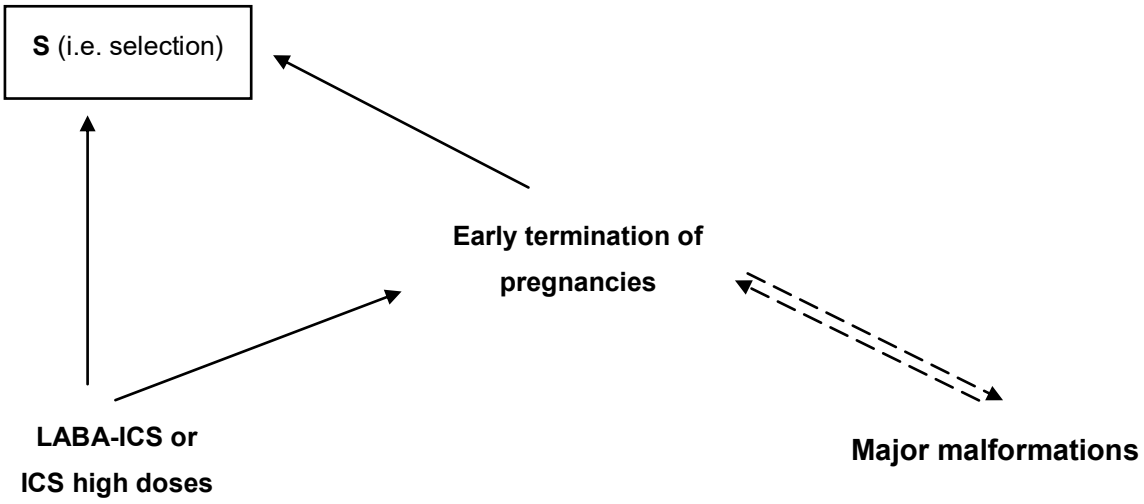

Fig. 6.3.1 Directed acyclic graph of a potential selection bias, first scenario

In the second scenario (see DAG in Fig. 6.3.2), another type of selection bias might have arisen, namely index event bias or collider-stratification bias.[277,278] In the case of the presence of an unmeasured variable(s) that is associated with both early termination of pregnancy and major malformations (e.g. maternal tobacco smoking, illicit drug use or a genetic factor), a potential index event bias could have occurred. In such case, a biasing path could have been formed leading to erroneous results (see DAG in Fig. 6.3.2). The direction and magnitude of the bias is difficult to anticipate since they will depend on the characteristics of the unmeasured variable(s) and its strength.

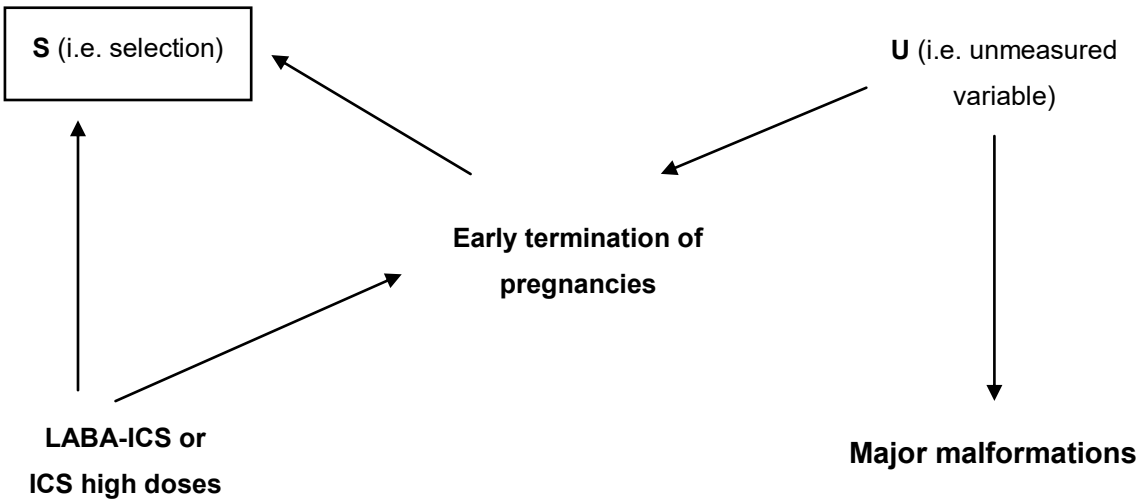

Fig. 6.3.2 Directed acyclic graph of a potential selection bias, second scenario

Information bias

Information bias occurs as a result of systematic differences in the way data on exposure, outcome, or potential confounders are obtained from the study groups.[236,270] Misclassification is one of the common forms of information bias, which can be differential or non-differential.[236,270,276] In retrospective cohort studies, in which information is obtained from past records, differential misclassification could be present if the quality and accuracy of information obtained is different among exposed and non-exposed persons.[236]

The outcome assessments (i.e. cases of major congenital malformations) were identified using diagnoses codes recorded in the RAMQ and MED-ECHO databases which were not specifically validated for this study, but validation was performed earlier in a separate study[61] and the outcome assessment was made independently of the exposure status of the mother. A possible detection bias[236,279] could have occurred in scenarios where women using asthma treatments in our study (i.e. LABA and ICS) have been systematically exposed to more intense follow-up during and after pregnancy, leading to higher detection rates of congenital malformations. This bias – if present – will most likely be non-differential (i.e. dilutes the true effect towards the null), unless more intense follow-up is provided for one treatment group over the other (e.g. high doses of ICS users over LABA plus medium doses of ICS). No evidence exists on such differential follow-up of asthmatic women during/after pregnancy and future research on this question is warranted. Non-differential misclassification generally dilutes the true effect towards the null, causing underestimation of the OR.[236,270,276] Regarding the non-significant result with the severe asthmatic subcohort, non-differential misclassification might have also played a role to prevent detecting an increased risk of congenital malformations.

Regarding the exposure assessment, the use of medications was measured using medication claims, which might not reflect their actual intake. Moreover, we considered maternal LABA exposure as dichotomous (i.e., exposed or not exposed during the first trimester) because in practice, the dose prescribed varies little between patients. This definition might have diluted the exposure because not all women will adhere fully (100%) to their LABA prescription and this could have contributed to an underestimation of the impact of the LABA-ICS combination on the risk of major malformations. Another

limitation concerning the use of the RAMQ database is that it doesn't record medications dispensed in hospitals, which may include oral corticosteroids, and this could have underestimated their use during pregnancy.

In our methodologic study, a possible detection bias could have occurred when women with asthma are compared to non-asthmatic women, since asthmatic women might more often undergo medical consulting and examination, both for themselves and their newborns, leading to more cases being detected among asthmatic women and biasing the results away from the null. Also in the methodologic study, we used a definition of asthma which was previously validated (see subsection 6.3.2.1). However, using a validated but not 100% accurate operational definition for asthma (i.e. with a sensitivity of 83.8% and a specificity of 76.5%) could have led to the incorrect classification of some asthmatic women as non-asthmatic and vice versa. Generally, the non-differential misclassification of exposure due to imperfect sensitivity and specificity in the presence of 2 exposure categories lead to a bias towards the null.[236]

In the article on the case ascertainment definitions of major malformations, the accuracy of the diagnoses in a hospital database is expected to be greater than in a medical claims database maintained mainly for billing purposes due to active and prospective data entry by trained medical archivists. The recording of all diagnoses in the medical claims database, including suspected and confirmed cases, could lead to false-positive cases. However, there is no reason to believe that the recording was differential between asthmatic and non-asthmatic women, reducing this potential bias towards the null. In this article, the lack of a gold standard prevented us from estimating the PPV or the NPV for the different case ascertainment definitions.

Confounding bias

Confounding is believed to be present when a spurious association appears due to the sharing of common causes.[267] The result is an observed association different from the true effect. When confounding occurs, the factor could be the alternative reason behind the association – or part of the association – observed between the exposure and the outcome.[236,270,276]

In the article on LABA-ICS combination versus ICS monotherapy, we used multivariate regression models to adjust the ORs for several confounding variables (see statistical analysis section in the manuscript). However, there was also a possibility of residual confounding arising from unmeasured risk factors for congenital malformations, such as cigarette smoking, maternal obesity, over-the-counter medications, and some other environmental teratogens.[160] As previously mentioned, we minimized the effect of the confounding by indication by performing the primary analysis within subcohorts of women with similar levels of asthma severity and through adjusting for baseline severity markers (including exacerbation of asthma and SABA doses/week three months before pregnancy). However, because there was no randomization of the treatment at the beginning, we cannot be sure that there was no residual confounding. Another possible source of residual confounding is the absence of information on the provider classification of asthma severity, since this variable is not recorded in the databases.

Other limitations

All studies using repeated statistical analysis simultaneously in one population to assess several drug exposures are subject to multiple comparisons problems and inference error, resulting in statistically significant P-values by chance alone.[236,280] As usual in studies of drug safety, we used a P-value <0.05 as the level for statistical significance, even if several comparisons were performed. We didn't adjust for multiple comparisons in our study. However, we did not report significant associations in our study, so such limitation (i.e. significant associations due to chance alone) is not applicable to our results.

In the article on LABA-ICS combination versus ICS monotherapy, since our objective was to examine the safety of two treatment regimens with similar indications, we cannot exclude the possibility that the use of either high-dose ICS or LABA is independently associated with a higher risk of major malformations than no use of these medications. However, such comparisons (use versus no use) are of lesser clinical relevance since not treating a woman who requires high-dose ICS or the addition of LABA to a lower-dose ICS to control her asthma during pregnancy is clearly not a recommended treatment option.

In the methodologic study, it is unlikely that one maternal exposure (i.e. asthma) will result in the increase of all major malformations categories. We used all major malformations combined only as an empirical example that provided the largest number of cases and the capacity to compare with previous studies. However, using all major malformations – and not system-specific malformations – could have diluted the effect of asthma on major malformations and might have prevented us from observing greater effect estimates for specific malformations.

6.3.2.4 External validity

External validity refers generally to which extent a study's findings could be applied to other non-study populations (i.e. generalizability).[236,270,276,281] We used the Quebec Asthma and Pregnancy Database, which includes all pregnancies in all women with ≥ 1 asthma diagnosis in the 2-year period preceding one of their deliveries and all pregnancies of a 4-times-larger random sample of other women who delivered between January 1, 1990 and March 31, 2010 in Quebec. The database includes 583,071 pregnancies, representing about 35% of all births in the province in this period of time and providing an exceptional external validity.[260]

In a recent study by our research team, the combination of maternal asthma and low socioeconomic status was shown to have a synergic effect on the prevalence of major congenital malformations.[282] In that study, the prevalence of major congenital malformations was 17% higher among asthmatic women compared to non-asthmatic women and drug insurance status at the start of pregnancy, which is considered a surrogate measure of socioeconomic status[283], modified the association between maternal asthma and major congenital malformations.[282] The prevalence of major congenital malformations was 42% higher among publicly insured asthmatic women with social welfare compared to 10% among publicly insured women without social welfare and 13% among privately insured women.[282]

In the article on LABA-ICS combination versus ICS monotherapy, our cohort underrepresents women with a higher socioeconomic status. This is because the database included only women covered by the RAMQ public drug insurance which includes women receiving social assistance and middle class working women. However, this under

representation might limit the study generalizability only if socioeconomic status is believed to be an effect modifier for the association between the exposure in our study (i.e. the choice of LABA-ICS combination or ICS monotherapy in higher doses) and major congenital malformations. Unlike the association between asthma and major malformations, there is currently no published data that indicates that the socioeconomic status could modify the effect of these two comparable treatment regimens. Therefore, further investigation into this area is recommended. Nevertheless, it is possible that since LABA-ICS combination regimen is generally more costly than ICS monotherapy in higher doses, women with lower socioeconomic status might use it only when their asthma reaches exceedingly severe levels. On the other hand, high socioeconomic status women could have access to this costly regimen regardless of their severity asthma levels. This could have led to the presence of more severe asthmatic women with low socioeconomic status in the higher dose ICS groups and less severe asthmatic women with higher socioeconomic status in the LABA-ICS groups. Since more severe asthma and low socioeconomic status are both associated with greater risk of congenital malformations, we might have overestimated the risk of congenital malformations among women treated with higher ICS doses.

In the methodologic study, despite the fact that we used the Quebec health databases, the results are mostly generalizable and can straightforwardly reflect to different settings where similar health administrative databases are available. However, it is important to point out that we did not have access to a gold standard to compare it with our case ascertainment definitions, in order to formally assess the external validity of the definitions we developed. Also in this study, the association between asthma and major congenital malformations could have been affected by the socioeconomic status of the women in the cohort. However, the objective of the study was not to unbiasedly quantify this association but rather to examine the effect of the differences in the case ascertainment definitions of major malformations and how they impact the selected empirical example (i.e. maternal asthma-major malformations). In fact, the crude estimates (i.e. crude odds ratios) of the measured associations could suffice to reach valid conclusions.

6.3.3 Systematic procedure for the classification of proven and potential teratogens

The procedure we developed here is a systematic and updatable one, with objective components in most of its processes. The primary aim of this procedure is to provide lists of medications that are proven and potential teratogens that can be used in research. However, the lists provided have potential utility in other areas. Congenital malformations can arise when maternal exposure occurs above a threshold dose and at a critical time for the development of a specific fetal organ or system.[284] Because the majority of organs and systems develop in the first trimester – with the exception of the central nervous system – this is considered the period of highest fetal risk.[73] To maintain the consistency of the results, we considered the first trimester as the period of major interest in our classifications. Further research to develop lists of medications causing developmental damage in the second and third trimesters is warranted.

In our lists, different medications in the same list might have different magnitudes of teratogenic risk, ranging from low to high. The lack of complete evidence about all the medications on the lists precludes any additional valid subclassification. Therefore, future research into this topic is recommended. The objectivity of the classification may have been compromised in instances in which expert opinion was required. However, we minimized this by blinding the reports of the experts and with the consensus process. The agreement between the experts' opinions in this study was good. To perform and provide accurate classification, the experts have used a standard and recognized criteria for proof of human teratogenicity, the criteria suggested by Shepard.[219]

In our methodology, TERIS database was not searched for potential teratogens but rather used for verification and classification of medications in Step 2. The main reasons are that TERIS is searchable only by the magnitude of the teratogenic risk rating and some agents have more than one magnitude of teratogenic risk rating. For example, tetracycline has a risk rating for dental staining and another one for malformations. Also, in the TERIS database, there are more than 1200 agents that have their risk rating as "UNDETERMINED". Therefore, when we conducted a search using "UNDETERMINED" as risk rating, the search-results box showed a message stating that there are too many results to show (i.e. impossible search). Moreover, the TERIS scheme that we developed use the "quality & quantity of data" ratings first then the "magnitude of teratogenic risk"

rating second, in order to classify the medications, and at this time it is not possible to do a search using the "quality & quantity of data" ratings in TERIS database. Also, there was a very slight chance that a medication could be present in TERIS but not in Briggs et al. book.

Reprotox and Shepard's Catalog of Teratogenic Agents are well-established teratogen information resources that we thought of including in the 2-Step procedure.[197,233] However, neither Reprotox nor Shepard's Catalog of Teratogenic Agents have risk ratings or classifiable index as Briggs et al. reference book or the TERIS database. Therefore, in order to make the search and classification process systematic and reproducible, we chose to use them only when the experts classified the medications on their own and during their consensus meeting. Briggs et al. book was chosen first as it provides the most exhaustive list of medications with classifiable index, and TERIS has ready-to-use risk ratings that we used to develop the TERIS scheme. Neither Reprotox nor Shepard's Catalog of Teratogenic Agents have such ratings. This way, we have tried to make the procedure systematic and easy to reproduce by other researchers as well. We used the pregnancy recommendations given by Briggs et al. in our procedure (Step 1), but some potentially unsafe medications might have been included under the pregnancy recommendation "compatible-maternal benefit >> embryo-fetal risk" and were consequently not verified by our expert. However, Step 1 also included screening other references, in which such medications were cited. Because the literature is rapidly expanding, there might still be medications that were missed or of which we were unaware at the time we finalized the current report.

6.4 Research significance and clinical implications

Implications for practice

Through conducting our systematic review on beta$_2$-agonists and perinatal outcomes, we found a larger body of knowledge on salbutamol compared to other SABA, and that adds to the evidence of its safety. It is difficult to conclude on the safety of other SABA (i.e. fenoterol and terbutaline), so we recommend that practitioners prescribe salbutamol for pregnant women in concordance with the guidelines.[5,14] Regarding LABA, evidence of increased risk of specific congenital malformations exists, but it is difficult to interpret this association as causal since part of the observed risk could be attributable to

the severity of asthma. Until this observation is reproduced in other large studies, it is difficult to make a clear recommendation, and the current guidelines should be followed.

The non-significant results we observed in several studies should be interpreted with caution since the majority of the negative studies were underpowered to detect clinically significant effects. The outcomes of our review are easily transferrable to physicians and specialists for the management of asthma during pregnancy. Our results could be very useful in adding to the physicians' trust in SABA as a quick relief medication and solve some benefit-risk questions and fears.

Our article on LABA-ICS combination versus ICS monotherapy in higher doses has a significant potential impact on the clinical practice of asthma management during pregnancy. The goal of asthma therapy – as the guidelines recommend – is to maintain optimal control of asthma symptoms and prevent acute asthma exacerbations. Among the important clinical decisions that physicians must make if asthma cannot be controlled with a low dose of ICS during pregnancy is whether to prescribe LABA to supplement the current dose of ICS or to increase the dose of ICS. However, it is still unknown whether it is safer for the newborn to increase the dose of ICS during pregnancy or to add a LABA. Our study has focused specifically on answering that question.

The results we presented add essential evidence-based knowledge that could reinforce the confidence of clinicians in prescribing LABA-ICS combination to keep the mothers' asthma under control, especially with the higher risk of congenital malformations with high doses of ICS monotherapy observed in earlier reports.[285] This could be part of a larger therapeutic strategy endorsed by health professionals and decision makers to provide better maternal care for asthmatic pregnant women. Such strategy would focus on keeping asthma under control throughout the pregnancy period and minimizing the exacerbations risk. The results are encouraging as well for the asthmatic women, motivating them to continue taking their asthma medications when required to control their asthma symptoms during pregnancy, increasing the likelihood of healthy pregnancies and newborns.

Implications for research

The article on LABA-ICS combination versus ICS monotherapy in higher doses represent a model for comparative effectiveness and safety research in the field of maternal

asthma treatments. Researchers can benefit from adapting our methodology – including the analytical methods and the subgroup analysis – to other research questions in this field. The major advantages are minimizing the confounding by indication and severity while providing results that are readily transferable to clinical practice.

In our methodologic article, we revealed how case ascertainment definitions had a considerable impact on the prevalence of major malformations, but a small influence on the aORs. Based on our research experience using large computerized administrative health databases from Quebec in the field of congenital malformations, we recommended the case ascertainment definition with ≥ 1 hospitalization diagnosis combined with the TCMC classification method, since it has the least chance of including misclassified and false positive cases. The detailed results of our study have direct implications for researchers working with Canadian health databases, but the main underlying results are transferrable to any computerized health database on congenital malformations. For researchers in this field, our results are highly valuable, especially on the association between asthma (as empirical example) and major malformations since we couldn't locate previous studies investigating the effect of several case ascertainment definitions on the measure of association. These results could assist in guiding future research on congenital malformations and the comparative effectiveness and safety of drug therapies during pregnancy.

We developed a systematic and updatable procedure for the classification of medications proven and potentially teratogenic when used during the first trimester of pregnancy. The objective was to provide lists that can be used primarily for research. The lists and the procedure itself can be of significant value for researchers in several fields, including teratology, perinatology, and reproductive epidemiology. We proposed ways through which our lists can be effectively used (e.g., exclusion of mothers exposed to them or by using various statistical adjustment techniques), however their use is not limited to those techniques. Our procedure has identified a large number of medications that were not reported in similar previous reports. Importantly, we identified a broad list of potentially teratogenic medications, which is unprecedented. Other areas of potential utility for our lists include screening medications that require high-priority postmarketing surveillance, and guiding future research into medications that require further investigation in animal models.

6.5 Future research

For future research on SABA and LABA and perinatal outcomes, we suggest updating the systematic review we published to include the most recently published studies (i.e. published after 1 January, 2013). We also recommend including studies that examined comparable treatment regimens in the systematic review, as the one we conducted and included in the current thesis. Future reviews might also consider performing meta-analysis of drug-specific effects from several well-conducted studies. In the field of maternal asthma treatments and congenital malformations, future studies should be large enough to be able to compare equivalent treatment regimens, or to compare different molecules of one class in order to minimize confounding by asthma severity. Additional large studies on treatment regimens that share similar indications should be the focus of future projects. An interesting question that complement our research would be: does the safety results on LABA-ICS combination and ICS monotherapy in higher doses hold for different system-specific malformations or not. This question requires a larger sample size than the one we had obtained and is highly justified for future research efforts.

Further research is needed to find solutions for some additional related questions that are equally urgent. More comparative effectiveness research is needed to identify the most effective and safest treatment regimens for maternal asthma among different subgroups of pregnant women. There is currently no data that support one treatment regimen over the other among specific patients' subgroups, including for example obese and overweight vs non-obese patients. Additional evidence in this area will be of high value. Moreover, similar knowledge is highly required for the different asthma phenotypes. Another area of scarce evidence in the comparative effectiveness of maternal asthma treatment regimens is the difference in adherence rates for patients on different comparable treatment regimens.

Future research on the case ascertainment definitions of major malformations could explore the associations between other maternal exposures and system-specific categories of congenital malformations (e.g. the association between maternal diabetes and cardiac defects). Moreover, formal assessment of the validity of the proposed case ascertainment definitions is certainly needed. We acknowledged the presence of some major congenital

malformations that could be debated to reflect minor malformations or less serious major malformations. We recommend the developing of a stricter case ascertainment definition (as a new classification method) which can be compared and validated as well in the validation study in the future.

We suggest that researchers endorse the teratogenic and potential teratogenic medications lists we presented for their epidemiologic research that include any first trimester exposure. Future work based on the new edition of Briggs et al. (2015) could be valuable, beside the already established method to update the medications lists. Further research to develop lists of medications causing developmental damage in the second and third trimesters is warranted. Also a comparison between the risk ratings for teratogenic medications in Briggs et al. book and TERIS database. A major drawback with lists is that there is no formal assessment of the severity or frequency of the specific malformation or the conditions under which the adverse effect occurred. Including this information will be valuable for future research. Moreover, it is highly recommended to expand the lists we proposed to cover data on which drugs cause teratogenicity due to their pharmacological mechanism of action, which ones cause teratogenicity only at high doses, and the types of malformations found to be associated with each teratogen.

6.6 Conclusion

The studies presented in the current thesis were conducted to achieve an ultimate objective, which is to examine the comparative safety of two common treatment regimens for maternal asthma during the first trimester of pregnancy. Our research program has expanded to cover other related and equally important areas.

In the first part, the results of the systematic review on beta$_2$-agonists and perinatal outcomes showed a larger body of knowledge on salbutamol compared to other SABA, an added proof for its safe use during pregnancy. Studies on maternal use of LABA during pregnancy are relatively fewer and smaller in size, warranting further research on their association with different perinatal outcomes. Conducting larger comparative safety studies and meta-analysis of several well-designed studies are two future research prospects that we highly praise.

In the second part of this thesis, we used a large linkable health administrative databases from Quebec to answer two different questions. The results of the first study showed that the risk of major malformations did not differ when a combination therapy of LABA plus ICS or a higher dose of ICS monotherapy was used in the first trimester of pregnancy. These results are encouraging for the expecting mothers and carry major clinical relevance for the health professionals. It will be interesting to see similar results replicated by other researchers in future studies, and equivalent methodology being used to examine other perinatal outcomes.

The results of our third study revealed how different case ascertainment definitions of major malformations had a considerable impact on the prevalence of major malformations, but a small influence on the aORs. The study results could assist in guiding future research on congenital malformations and pharmacoepidemiologic studies on drug therapies during pregnancy. A comprehensive validation study with medical records as gold standard is strongly suggested for future research efforts.

In the last part we used reliable references and resources to develop a systematic and updatable procedure for the classification of teratogenic and potential teratogenic medications. The lists we developed effectively resolve the discrepancies and contradictions of teratogens lists previously published in the literature. The lists are to be used primarily for research, with significant value for researchers in teratology, perinatology, and reproductive epidemiology. Regular updating for the lists to include newly published evidence and further expansion to include additional teratogenicity details is interesting to see in future work.

In conclusion, the results presented in the current thesis have a significant added value to the published evidence on asthma treatments during pregnancy, helping clinicians and mothers to choose the optimal therapeutic regimen to keep asthma under control during pregnancy. The thesis' results added also constructive knowledge that could have remarkable research implications. The major knowledge gaps that we addressed – on indication bias, congenital malformations ascertainment and teratogenic medications – provided valuable evidence that is directly transferable to researchers in teratology, pharmacoepidemiology and other related research fields.

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Appendix A

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NHS
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PROSPERO International prospective register of systematic reviews

Beta-2-agonists during Pregnancy and Perinatal Outcomes: a Systematic Review with Focus on Statistical Power

Sherif Eltonsy, Fatima-Zohra Kettani, Lucie Blais

Citation

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Review question(s)

To perform a review of the existing literature regarding the use of beta-2-agonists during pregnancy and the risk of adverse perinatal outcomes, with particular focus on study power.

we aim to summarize the existing human data on the impact of these medications on major and all congenital malformations, SGA, birth weight, LBW, gestational age, and preterm delivery.

We also aim to perform post-hoc power calculations to evaluate the capacity of each study to detect the associations under study.

Searches

We will search the following electronic bibliographic databases: PubMed, MEDLINE, EMBASE, The Cochrane Library, CINAHL, and Web of Science. No date restrictions are applied, and only articles in the English language will be included in the final pool of studies. Abstracts without supporting articles are not included.

Types of study to be included

No restrictions are applied on the study designs.

Condition or domain being studied

Asthma during pregnancy, Perinatal outcomes

Participants/ population

Inclusion: Pregnant women exposed to Beta-2-agonists during pregnancy

Exclusion: Men, Non-pregnant women, and pregnant women not exposed to beta-2-agonists

Intervention(s), exposure(s)

beta-2-agonists have a crucial role in asthma management during pregnancy. Short-acting beta-2-agonists (SABA) are used as quick relief medications for all asthma types (mild, moderate, or severe), while long-acting beta-2-agonists (LABA) are used in cases of moderate and severe persistent asthma, in

combination with low or medium doses of inhaled corticosteroids.

The exposure to SABA and LABA during pregnancy is the objective of this review.

Comparator(s)/ control

Beta-2-agonists users will be compared against non-users (asthmatics), non-asthmatics, and other asthma medication users.

Outcome(s)

Primary outcomes

Major and all congenital malformations, small for gestational age (SGA), birth weight, low birth weight (LBW),gestational age, and preterm delivery

Secondary outcomes

None

Data extraction, (selection and coding)

The primary search was conducted by one author (SE), while a second confirmatory independent search was performed by a second author (FZK). All studies identified in the search were independently reviewed by two co- authors and the study selection was made independently by two co-authors (SE and FZK). Data extraction and post- hoc power calculations were first performed by one author (SE). An independent data extraction and post-hoc power calculation were performed by a second author (FZK) under unmasked condition. Discrepancies were resolved by consensus

Data retrieved from each study included the study reference, the design, the source of data, the timing of exposure, the type of beta2-agonists, the definition of the reference group, the sample size of the exposed and unexposed groups, the reported proportions or means and standard deviations for the outcomes in the exposed and unexposed groups, the effect size (crude or adjusted relative risk (RR), odds ratio (OR), or mean difference (MD)), and the p- value or 95% confidence interval (CI) associated with the effect size. In studies that did not report the effect size, a crude RR, OR, or MD was calculated when sufficient information was provided.

We performed a post-hoc power calculation for each study reporting non-statistically significant results to detect a RR of 1.5, a mean difference in the birth weight of 500 g, or a mean difference in gestational age of one week to establish a comparison between studies. The power calculations were based on t-tests for MD and on the test for the difference between two independent proportions for RR and OR. A type I error of 0.05 was used for power calculations; all calculations were performed using PASS 2008 interface of NCSS software.

Risk of bias (quality) assessment

All studies included in this review to be independently reviewed by two co-authors, and data extraction performed by the two authors independantly.

Strategy for data synthesis

We will provide a narrative synthesis of the findings from the included studies, including the design, the source of data, the timing of exposure, the type of beta2-agonists, the definition of the reference group, the sample size of the exposed and unexposed groups, the reported proportions or means and standard deviations for the outcomes in the exposed and unexposed groups, the effect size (crude or adjusted relative risk (RR), odds ratio (OR), or mean difference (MD)), and the p-value or 95% confidence interval (CI) associated with the effect size.. We will provide summaries of intervention effects for each study, and post-hoc power calculations for each study. We anticipate that there will be limited scope for meta-analysis because of the range of different outcomes measured across the small number of existing studies.

We will calculate the power of each study (excluding those reporting significant results) to detect a RR of 1.5, a mean difference in the birth weight of 500 g, or a mean difference in gestational age of 1 week to

establish a comparison between studies. The power calculations are based on t-tests for MD and on the test for the difference between two independent proportions for RR and OR. A type I error of 0.05 will be used for power calculations, and all calculations will be performed using PASS 2008 interface of NCSS software.

Analysis of subgroups or subsets
None planned

Dissemination plans
A paper will be submitted to a leading journal in this field.

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Anticipated completion date
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Conflicts of interest
L. Blais is the recipient of a Salary Award from the Fonds de la recherché en santé du Québec (FRSQ) and co-chairs the Endowment Pharmaceutical Chair AstraZeneca in Respiratory Health. L. Blais has received research support from AstraZeneca, Amgen, and GlaxoSmithKline. S. Eltonsy has declared that he has no conflict of interest. FZ. Kettani has declared that she has no conflict of interest.

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Subject indexing assigned by CRD

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Completed but not published

Date of registration in PROSPERO
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Stage of review at time of this submission	Started	Completed
Preliminary searches	No	Yes
Piloting of the study selection process	No	Yes
Formal screening of search results against eligibility criteria	No	Yes
Data extraction	No	Yes
Risk of bias (quality) assessment	No	No
Data analysis	No	Yes

Appendix B

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE

CASE CONTROL STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

Selection

1) Is the case definition adequate?
   a) yes, with independent validation ✸
   b) yes, eg record linkage or based on self reports
   c) no description

2) Representativeness of the cases
   a) consecutive or obviously representative series of cases ✸
   b) potential for selection biases or not stated

3) Selection of Controls
   a) community controls ✸
   b) hospital controls
   c) no description

4) Definition of Controls
   a) no history of disease (endpoint) ✸
   b) no description of source

Comparability

1) Comparability of cases and controls on the basis of the design or analysis
   a) study controls for_____(Select the most important factor.) ✸
   b) study controls for any additional factor ✸ (This criteria could be modified to indicate specific control for a second important factor.)

Exposure

1) Ascertainment of exposure
   a) secure record (eg surgical records) ✸
   b) structured interview where blind to case/control status ✸
   c) interview not blinded to case/control status
   d) written self report or medical record only
   e) no description

2) Same method of ascertainment for cases and controls
   a) yes ✹
   b) no

3) Non-Response rate
   a) same rate for both groups ✹
   b) non respondents described
   c) rate different and no designation

COHORT STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability

Selection

1) Representativeness of the exposed cohort
   a) truly representative of the average_____(describe) in the community ✹
   b) somewhat representative of the average_____in the community ✹
   c) selected group of users eg nurses, volunteers
   d) no description of the derivation of the cohort

2) Selection of the non exposed cohort
   a) drawn from the same community as the exposed cohort ✹
   b) drawn from a different source
   c) no description of the derivation of the non exposed cohort

3) Ascertainment of exposure
   a) secure record (eg surgical records) ✹
   b) structured interview ✹
   c) written self report
   d) no description

4) Demonstration that outcome of interest was not present at start of study
   a) yes ✹
   b) no

Comparability

1) Comparability of cohorts on the basis of the design or analysis
   a) study controls for_____(select the most important factor) ✹
   b) study controls for any additional factor ✹ (This criteria could be modified to indicate specific control for a second important factor.)

Outcome

1) Assessment of outcome
   a) independent blind assessment ✹
   b) record linkage ✹
   c) self report
   d) no description

2) Was follow-up long enough for outcomes to occur
   a) yes (select an adequate follow up period for outcome of interest) ✹
   b) no

3) Adequacy of follow up of cohorts
   a) complete follow up - all subjects accounted for ✹
   b) subjects lost to follow up unlikely to introduce bias - small number lost - >_____% (select an adequate %) follow up, or description provided of those lost) ✹
   c) follow up rate <_____% (select an adequate %) and no description of those lost
   d) no statement

CODING MANUAL FOR CASE-CONTROL STUDIES

SELECTION

1) Is the Case Definition Adequate?

a) Requires some independent validation (e.g. >1 person/record/time/process to extract information, or reference to primary record source such as x-rays or medical/hospital records) ☆
b) Record linkage (e.g. ICD codes in database) or self-report with no reference to primary record
c) No description

2) Representativeness of the Cases

a) All eligible cases with outcome of interest over a defined period of time, all cases in a defined catchment area, all cases in a defined hospital or clinic, group of hospitals, health maintenance organisation, or an appropriate sample of those cases (e.g. random sample) ☆
b) Not satisfying requirements in part (a), or not stated.

3) Selection of Controls

This item assesses whether the control series used in the study is derived from the same population as the cases and essentially would have been cases had the outcome been present.

a) Community controls (i.e. same community as cases and would be cases if had outcome) ☆
b) Hospital controls, within same community as cases (i.e. not another city) but derived from a hospitalised population
c) No description

4) Definition of Controls

a) If cases are first occurrence of outcome, then it must explicitly state that controls have no history of this outcome. If cases have new (not necessarily first) occurrence of outcome, then controls with previous occurrences of outcome of interest should not be excluded. ☆
b) No mention of history of outcome

COMPARABILITY

1) Comparability of Cases and Controls on the Basis of the Design or Analysis

A maximum of 2 stars can be allotted in this category

Either cases and controls must be matched in the design and/or confounders must be adjusted for in the analysis. Statements of no differences between groups or that differences were not statistically significant are not sufficient for establishing comparability. Note: If the odds ratio for the exposure of interest is adjusted for the confounders listed, then the groups will be considered to be comparable on each variable used in the adjustment.

There may be multiple ratings for this item for different categories of exposure (e.g. ever vs. never, current vs. previous or never)

Age =☆ , Other controlled factors = ☆

EXPOSURE

1) Ascertainment of Exposure

Allocation of stars as per rating sheet

2) Non-Response Rate

Allocation of stars as per rating sheet

CODING MANUAL FOR COHORT STUDIES

SELECTION

1) Representativeness of the Exposed Cohort

Item is assessing the representativeness of exposed individuals in the community, not the representativeness of the sample of women from some general population. For example, subjects derived from groups likely to contain middle class, better educated,

health oriented women are likely to be representative of postmenopausal estrogen users while they are not representative of all women (e.g. members of a health maintenance organisation (HMO) will be a representative sample of estrogen users. While the HMO may have an under-representation of ethnic groups, the poor, and poorly educated, these excluded groups are not the predominant users users of estrogen).

Allocation of stars as per rating sheet

2) Selection of the Non-Exposed Cohort

Allocation of stars as per rating sheet

3) Ascertainment of Exposure

Allocation of stars as per rating sheet

4) Demonstration That Outcome of Interest Was Not Present at Start of Study

In the case of mortality studies, outcome of interest is still the presence of a disease/ incident, rather than death. That is to say that a statement of no history of disease or incident earns a star.

COMPARABILITY

1) Comparability of Cohorts on the Basis of the Design or Analysis

A maximum of 2 stars can be allotted in this category
Either exposed and non-exposed individuals must be matched in the design and/or confounders must be adjusted for in the analysis. Statements of no differences between groups or that differences were not statistically significant are not sufficient for establishing comparability. Note: If the relative risk for the exposure of interest is adjusted for the confounders listed, then the groups will be considered to be comparable on each variable used in the adjustment.
There may be multiple ratings for this item for different categories of exposure (e.g. ever vs. never, current vs. previous or never)
Age = ☆ , Other controlled factors = ☆

OUTCOME

1) Assessment of Outcome

For some outcomes (e.g. fractured hip), reference to the medical record is sufficient to satisfy the requirement for confirmation of the fracture. This would not be adequate for vertebral fracture outcomes where reference to x-rays would be required.

a) Independent or blind assessment stated in the paper, or confirmation of the outcome by reference to secure records (x-rays, medical records, etc.) ☆
b) Record linkage (e.g. identified through ICD codes on database records) ☆
c) Self-report (i.e. no reference to original medical records or x-rays to confirm the outcome)
d) No description.

2) Was Follow-Up Long Enough for Outcomes to Occur

An acceptable length of time should be decided before quality assessment begins (e.g. 5 yrs. for exposure to breast implants)

3) Adequacy of Follow Up of Cohorts

This item assesses the follow-up of the exposed and non-exposed cohorts to ensure that losses are not related to either the exposure or the outcome.

Allocation of stars as per rating sheet

Appendix C

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	7
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	8
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	8
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	9
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	9
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	9
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	10

Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	10
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	10
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	10
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	11
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	NA
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	NA

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	NA
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	11
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	11, Fig.1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	35-45
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	34
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	35-45

Synthesis of results	21	Present the main results of the review. If meta-analyses are done, include for each, confidence intervals and measures of consistency	11-18, 35-45
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	NA
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	35-45
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	18-24
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	24
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	25
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	2

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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