Asthma and Pregnancy: Possible to prevent complications?

- With Special reference to the impact of obesity and type of airway inflammation

Zarqa Ali

This review, supplemented by four published papers, has been accepted as a thesis by University of Copenhagen 2th of June 2017 and defended on 28th of June 2017.

Tutors:
Charlotte Suppli Ulrik, Lisbeth Nilas.

Official opponents: Celeste Porsbjerg,, Cecilie Svanes, Francisco Gómez Real

Correspondence:
Department, Department of Pulmonary Medicine
Hvidovre Hospital

E-mail: Zarqa_ali@hotmail.com

Dan Med J 2017;64(12):B5428

The 4 original papers

Study 1: Ali Z, Nilas L, Ulrik CS. Determinants of low risk of asthma exacerbation during pregnancy. Clin Exp Allergy 2017;00:1–7. (Epub)

Study 2: Ali Z, Nilas L, Ulrik CS. Excessive gestational weight gain in first trimester is a risk factor for exacerbation of asthma during pregnancy: A prospective study of 1283 pregnancies. The Journal of allergy and clinical immunology May 24 2017. (Epub)

Study 3: Ali Z, Nilas L, Ulrik CS. Postpartum airway responsiveness and exacerbation of asthma during pregnancy – a pilot study. Journal of Asthma and Allergy 2017:10 261–267. (Epub)

Study 4: Ali Z, Nilas L, Ulrik CS. Low risk of adverse obstetrical and perinatal outcome in pregnancies complicated by asthma: A case control study. Respir Med 2016;120:124-130

Introduction

Asthma is a serious global health issue affecting around 300 million individuals worldwide (1). The prevalence of asthma has increased in the recent decades and is still increasing in many developing countries (1). In Denmark, the prevalence varies from 12% in children (2) to 6-8% in adults (3-5). In adults, the prevalence of asthma is higher among females than in males (6, 7), and asthma is the most common chronic disease in pregnant women (8).

Asthma is characterized by periodic worsening of symptoms, and may also in patients with milder disease lead to acute exacerbations. Exacerbations during pregnancy have been associated with an unfavorable pregnancy outcome, and by that making asthma a potential serious medical condition during pregnancy (9). Pregnant women with asthma are less likely to be prescribed oral corticosteroids (OCS) for acute exacerbations (10-12), and the treatment delay is longer (10), compared to non-pregnant women with asthma. This is in contrast to the knowledge, that prompt treatment of an exacerbation is important, as the potential reduction in the fetal oxygen supply during a severe exacerbation represents a larger risk for the fetus than the administration of relevant anti-asthma drugs (13).

Based on our current knowledge, optimal treatment of asthma during pregnancy and prevention of exacerbations is of outmost importance for the health of both the mother and the child. It is recommended to monitor pregnant women with asthma every four to six weeks, although there is little evidence that this regimen improves pregnancy outcome or is beneficial for all pregnant women with asthma (1, 13).

The overall aim of the present thesis was to gain more knowledge about the interaction between asthma and pregnancy.

The specific research questions were

- to identify pregnancies with a low-risk of exacerbations during pregnancy
- to identify risk factors for exacerbations during pregnancy
- to compare the adverse pregnancy and perinatal outcomes in women with asthma monitored closely as recommended during pregnancy with women without asthma.

Background

1. Asthma

Asthma is a heterogeneous disease characterized by chronic airway inflammation and airway hyperresponsiveness leading to recurrent episodes of respiratory symptoms, including wheezing, breathlessness, tightness of the chest, and coughing (14). The severity of symptoms varies highly over time both between individuals and within the individual patient. The diagnosis of asthma is based on a history of typical respiratory symptoms together with an objective test demonstrating variable airflow obstruction, e.g. bronchodilator reversibility, diurnal peak flow variability and bronchial hyperresponsiveness (1).

1.1 Asthma exacerbation

According to the latest Global Initiative for Asthma (GINA) guidelines, an exacerbation is defined as a worsening in symptoms requiring a change in medication (1). Depending on the severity, an exacerbation can vary from breathlessness to respiratory failure requiring mechanical ventilation. Prevention of an exacerbation is important, as it constitutes a great risk to the patients. Even though an exacerbation is considered the most important outcome leading

to accelerated loss of lung function (15), and thus a worse prognosis (15), exacerbations have only been used as a primary outcome variable in clinical trials for the past two decades (16). The classification of the severity of an asthma exacerbation has varied between studies, and there is no generally accepted definition of an exacerbation (17). In 2003, a Task Force established by European Respiratory Society (ERS) and American Thoracic Society (ATS) provided consensus recommendations on standardized definitions for assessing asthma exacerbations in clinical practice and clinical trials. Severe exacerbations were defined as events requiring urgent action to prevent a serious outcome, such as hospitalization or death from asthma, and moderate exacerbations as less severe events requiring immediate change in treatment. However, no definitions were provided for mild exacerbations, because the worsening of symptoms or flow rates may only be slightly outside the normal range of variation for the individual patient and may reflect transient loss of asthma control rather than the early stages of severe exacerbation (18).

The GINA guidelines defines an asthma exacerbation as an acute or subacute episode of progressive worsening of asthma symptoms and by a decrease from baseline in objective measures of pulmonary function, such as peak expiratory flow rate and FEV1 (1). As the lung function is not always measured at the time of an exacerbation, the majority of clinical and epidemiological studies define a severe exacerbation according to ERS/ATS guidelines (18).

2. Asthma and pregnancy

Asthma is the most common reason for being prescribed pharmacological maintenance therapy in pregnancy (19), and the most prevalent chronic disorder among Danish pregnant women (8).

2.1 Incidence and timing of asthma exacerbations during pregnancy

The general dogma is that one-third of women with asthma will experience a worsening, one-third an improvement, and one-third no changes in their asthma during pregnancy. These figures are derived from a prospective study in 300 women assessed by daily questionnaires and monthly spirometry (20). The study showed that asthma worsened in 35% of the pregnant women, improved in 28%, and was unchanged in 33% (status undetermined in the remaining 4%). Exacerbations can occur at any time during pregnancy, but tend to cluster around late second trimester (21-23). Exacerbations during third trimester and, especially, worsening of symptoms during labor are rare (20, 24).

2.2 Risk factors for asthma exacerbations during pregnancy

The exacerbation rate increases with increasing asthma severity, and severe asthma is likely to be the most important risk factor for exacerbations during pregnancy (22, 23, 25). Schatz et al. (25) found that asthma exacerbations during pregnancy occurred in 13% of women classified with mild, 26% of women with moderate, and 52% of women with severe asthma. In line with this Murphy et al. (22) showed, that severe asthma exacerbations during pregnancy occurred in 8% of women with mild asthma, 47% of women with moderate asthma, and 65% of women with severe asthma.

Non-adherence with inhaled corticosteroids (ICS) is a major contributor to exacerbations also during pregnancy (21, 22, 26), and women, who do not regularly take ICS, have increased risk of visits to the emergency department (26). A descriptive web-based survey revealed that at least one-third of women were non-adherent with ICS during pregnancy, and 44% were concerned about how asthma medication would affect their baby (27). Fear of potential of teratogenic risks influence decisions about drug therapy in pregnancy, and is very likely to have negative impact on adherence with prescribed medication (28). Nonetheless, enrolment in an asthma management program during pregnancy improves adherence with controller medication (29).

Women inadequately treated with ICS during pregnancy have a higher risk of an asthma exacerbation during pregnancy, and adequate treatment with ICS reduces the risk of an exacerbation by more than 75% (30). In addition, a previous study has shown that approximately 25% of the general practitioners would stop treatment with controller medication in pregnant women with well-controlled asthma (31).

Other possible risk factors for exacerbation of asthma during pregnancy include maternal obesity (32) and smoking (33). Asthma exacerbations during pregnancy are more common and more severe in current and former smokers than in never smokers (33). Passive tobacco exposure in never-smokers is associated with impaired lung function, need for higher dose of ICS and a higher likelihood of uncontrolled asthma during pregnancy compared to never-smokers without tobacco exposure (34). There is some data indicating that overweight and obesity is associated with worsening of asthma in non-pregnant adults (35), and obesity has been associated with an increased risk of asthma exacerbations during pregnancy (32). Maternal obesity is also associated with increased risk of adverse pregnancy outcomes; including fetal anomalies, miscarriages, pre-eclampsia, gestational diabetes, cesarean delivery, and intrauterine fetal death (36, 37).

2.3 Effect of maternal asthma on pregnancy and perinatal outcomes

Previously published studies addressing pregnancy and perinatal outcomes in relation to asthma have reported conflicting results probably caused by substantial variations in study design, sample sizes, and adjustment for confounders. The first meta-analysis of available studies was published in 2011 and confirmed an increased risk of pre-eclampsia, preterm birth, low birth weight and small for gestational age infants among women with asthma compared to women without asthma (38). A later meta-analysis concluded that maternal asthma is associated with increased risk of caesarean section, gestational diabetes, hemorrhage, placenta praevia, and premature rupture of the membranes (39). Compared to women with good asthma control and no admissions for asthma during pregnancy, the incidence of preterm delivery was higher among women with poor asthma control and/or hospital admission for asthma during pregnancy (40). Furthermore, moderate and severe asthma, based on database indexes on the medication, has been associated with a higher risk of small for gestational age infants compared to mild asthma in an observational study (41). In line with this, women experiencing an asthma exacerbation during pregnancy have three times higher risk of having a low birth weight baby than women without exacerbations (42).

On the other hand, prospective cohort studies have found no significant relationship between asthma exacerbations during pregnancy and adverse pregnancy outcome such as preterm delivery, pre-eclampsia, and low birth weight in women with actively managed asthma (30, 38, 43, 44).

2.4 Treatment and monitoring of asthma during pregnancy

Management guidelines for pregnant women with asthma are generally the same as for non-pregnant women. Although there is a general concern about use of any medication during pregnancy,

the advantages of active treatment of asthma in pregnancy markedly overweigh the potential risks associated with the use of controller or reliever medication (45). The recommendations of a stepwise increase in medication intensity, and use of low-dose inhaled corticosteroids are the same during pregnancy than for other adults with asthma (13). Low-dose inhaled budesonide, i.e. a daily dose of 400 µg (metered dose) may be first choice based on the amount of available evidence (46). If step-up therapy is needed during pregnancy, it is recommended to increase to middle-ICS instead of, as in non-pregnant adults, to add-on long-acting beta-2 agonist (LABA) to low-dose ICS (46). Guidelines for management of asthma during pregnancy, are not based on evidence from controlled clinical trials. Regular monitoring of asthma every four to six weeks is recommended during pregnancy including assessment of asthma control (1, 13). Nonetheless, a substantial proportion of pregnant women with asthma have no exacerbations during pregnancy, and may not benefit from the recommended close monitoring during pregnancy. In order to individualize the intensity of asthma monitoring it is important to identify pregnancies with low risk of asthma exacerbation. However, our current knowledge of factors associated with low risk of an exacerbation during pregnancy is very limited (47).

Aim

The overall aim of the present thesis was to gain more knowledge of the interaction between asthma and pregnancy.

Consequently, four studies were performed with the following aims:

Study I

To identify determinants of pregnancies with low risk of asthma exacerbation during pregnancy

Study II

To investigate the impact of maternal pregnancy-related factors, including gestational weight gain, on the risk of exacerbation of asthma during pregnancy

Study III

To investigate potential differences in physiological and inflammatory characteristics between women with and without asthma exacerbations during pregnancy

Study IV

To compare adverse pregnancy and perinatal outcomes in women without asthma and women with asthma monitored closely.

Design, material and methods

The Management of Asthma during Pregnancy (MAP) program

The Management of Asthma during Pregnancy (MAP) program was established as a specialized service in 2007 and as a collaboration between the Department of Pulmonary Medicine and the Department of Gynecology/Obstetrics, Hvidovre Hospital. Hvidovre Hospital has the largest birth center in Denmark (around 7.000 deliveries annually, corresponding to 10% of infants born in Denmark). All pregnant women referred for delivery at Hvidovre Hospital are invited to participate in the MAP program, and those responding by mail (astmaoggraviditet@regionh.dk) are given an appointment at the outpatient clinic, Department of Pulmonary Medicine, by letter. A visit is planned every four weeks during pregnancy and the scheduled MAP-program ends with a follow-up visit three months post-partum. Unscheduled visits are planned if necessary.

Content of the visits

At the first visit details about case history, including tobacco exposure, allergies, familiar disposition to asthma and atopic diseases, present and previous asthma medication, exposure for pets and other triggers, occupational exposure, size of household, incl. siblings are obtained. The importance of adherence is addressed and instruction and training in device technique is provided if needed.

Spirometry, fractional exhaled nitric oxide (FENO), asthma-control and maternal body weight are assessed at the first visit and repeated at all subsequent visits. Body weight is measured without shoes and in light clothing at every visit.

Based on present level of asthma control, incl. symptoms, FENO and spirometry, treatment is adjusted in accordance with the algorithm later described by Powell et al. (48)

Inclusion criteria in the Map cohort

The MAP cohort is a prospective cohort study of women participating in the MAP program and fulfilling the inclusion criteria.

Inclusion criteria for the MAP cohort are

1) Diagnosis of asthma in accordance with the GINA-guidelines (1)

2) Current treatment with at least rescue bronchodilator

3) The first visit to the pulmonary outpatient clinic within the first 18 weeks of pregnancy

Women with asthma not fulfilling the inclusion criteria for the MAP cohort, i.e. the research program, are offered the same follow-up program as part of the clinical program.

The post-partum examination (Study III)

During an 11-month period, selected participants in the MAP study were invited, provided they fulfilled the criteria below, to an additional post-partum examination within the first nine months after delivery. The additional post-partum examination supplemented the preplanned visit three months post-partum with skin prick test with a panel of aeroallergens, body plethysmography, diffusing capacity, bronchial provocation test with mannitol, and induction of sputum.

Inclusion criteria for study III were:

1) Delivery between July 2015 and September 2016.

2) Prescribed controller medication, i.e. ICS, in early pregnancy

The control group (Study IV)

The control group in the case-control study (study IV) comprises the three first consecutive women giving birth at Hvidovre Hospital after each of the individual MAP participant.

Definitions

The severity of asthma was categorized as mild or moderate/severe based on the prescribed level of treatment according to the GINA guidelines (1). Mild asthma was defined as asthma that could be controlled by short-acting β2-agonist (SABA) as rescue medication with or without low dose inhaled corticosteroid (ICS) (according to treatment step 1 or 2 in GINA). Moderate/severe asthma was defined as asthma treated with any dose of ICS in combination with long-acting β2-agonist and/or leukotriene receptor antagonist with or without another add-on controller treatment (according to treatment step 3, 4 or 5 in GINA).

Asthma-control was assessed based on daytime asthma symptoms, night-time awakenings due to asthma, use of rescue medication, asthma-related activity limitation, spirometry, and the level of FENO. Well-controlled, partly controlled, and uncontrolled asthma was defined according to the GINA guidelines (1), and patients were classified as having clinically stable asthma provided they fulfilled the GINA criteria, and had a normal lung function (FEV1>80% predicted), and a FENO<25 ppb.

Severe asthma exacerbations were defined according to the ATS/ERS guidelines on asthma control and exacerbations (18)as exacerbations requiring hospital admission, emergency department treatment, and/or a rescue course of oral corticosteroids. Mild asthma exacerbations were defined as exacerbations managed by an increase in therapy, but not requiring oral corticosteroids. Smoking status was classified as current smokers (minimum one cigarette per day), ex-smokers(stopped before or when the current pregnancy was confirmed), or life-long non-smokers. Life-time tobacco exposure was estimated as pack-years: (number of cigarettes per day/20 x duration of smoking [years]). Exposure to environmental tobacco smoke (ETS)was defined as living with someone smoking at home (as smoking in work places, restaurants etc. is prohibited in Denmark).

Statistical analysis

Data were analysed using SAS EnterpriseGuide7.1. Continuous variables were analyzed using the two-tailed Student t-test. Binary variables were analyzed by the Chi-square test or Fisher-exact test, and results were given as odds ratios (OR) with 95% confidence intervals (CI).Mann-Whitney test was used to analyse not normally distributed data. A multiple logistic regression with backward elimination was carried out, using the ProcLogistic procedure in SAS. In study IV the response-dose ratio (RDR) was calculated as the percentage decline in FEV1afterthe last mannitol dose divided by the cumulative mannitol dose in milligrams. To meet normal distribution, log-transformed values of RDR were used, and an additional 1% was added to the percent decrease inFEV1toeliminate zero values. The association between airway responsiveness to mannitol expressed by the response-dose ratio (RDR), and the airway inflammation in sputum were analyzed using Pearson's correlation coefficient test (r), with-1 indicating a perfect negative correlation, +1 indicating a perfect positive correlation, and 0 indicating no correlation at all. In all the statistical analyses, a two-tailed p-value of ≤0.05 was considered significant.

Results

Study I

Over the 9-year study period (from 2007 to 2015) 1,283 pregnancies in 1,208 women aged 31±5 years were included in the MAP cohort. During the study period, 107 exacerbations were observed. In pregnancies with clinically stable asthma at the first visit only 5% (n=40) were complicated by an exacerbation, while the remaining 95% (n=839) experienced no exacerbations (p<0.001). Among women with no history of pre-pregnancy exacerbations, 4% (n=37) had an exacerbation during pregnancy (OR 0.22, 95% CI 0.14-0.35). Multiple logistic regression analysis revealed that no history of pre-pregnancy exacerbations, no prescription of controller medication, and clinically stable asthma at the first visit were independent determinants of pregnancies with a low risk of an exacerbation.

Study II

In 1.283 included pregnancies, the mean pre-pregnancy BMI was 24.2 (SD 4.5), first trimester GWG 5.1 kg (SD 3.4), and total GWG 12.5 kg (SD 5.8). The occurrence of an exacerbation of asthma during pregnancy was significantly related to first trimester gestational weight gain (GWG), and the impact of GWG on exacerbation risk was dose-dependent. The risk of an asthma exacerbation during pregnancy was low in women with a GWG less than 5 kg and increased with further weight gain.

Correspondingly women with total GWG more than 13 kg had higher risk of asthma exacerbation (OR 2.89 95% CI (2.18-3.83), p<0.0001) than women with weight gain less than 13kg.

Study III

During the 11-month study period, 104 women fulfilled the inclusion criteria, of whom 50 (48%) were examined postpartum within seven months after delivery. In 13 women, 16 exacerbations (8 mild and 8 severe) were observed during pregnancy, and three (6%) had more than one exacerbation during pregnancy.

There were no differences in the outcome of mannitol test (positive vs negative) in women with and without asthma exacerbations, however women with an exacerbation were more responsive to mannitol than women without an exacerbation (geometric mean PD15 82 vs 178 mg, p=0.04). Furthermore, there was a significant positive correlation between airway responsiveness to mannitol expressed as the response-dose ratio (RDR) and the neutrophil count in sputum (r = 0.44, p = 0.01).

Women without an asthma exacerbation during pregnancy were more likely to have a positive skin prick test compared to women with an exacerbation (86% vs 62%, OR 0.20, 95% CI 0.05-0.92, p=0.04).

Study IV

We included 938 pregnancies in women with asthma (i.e. cases) and 2,778 without asthma (i.e. controls). Compared to the controls, women with asthma were less often immigrants, and more often non-smokers and primiparous. The mean fetal weight was lower in women with asthma than in controls: 3379g (SD 571) versus 3438g (SD 561) (p=0.006), and women with asthma had increased risk of having a small for gestational aged (SGA) child (ORadj 1.31, 95% CI 1.12-1.55; p=0.001). Furthermore, maternal asthma was associated with higher risk of mild to moderate preeclampsia (ORadj 1.54, 95% CI 1.05-2.25; p=0.027) and gestational diabetes (ORadj 1.78, 95% CI 1.01-3.13; p=0.047).

Women with moderate/severe asthma had significantly increased risk of SGA infants (60% versus 53%, ORadj. 1.43, 95% CI 1.05-1.94; p=0.022).

Discussion

In short, we found that clinically stable asthma in early pregnancy, no history of pre-pregnancy exacerbations, and no prescribed controller medication are associated with pregnancies at low risk of an asthma exacerbation during pregnancy. Moreover, excessive gestational weight gain, airway responsiveness to mannitol, and non-atopy are risk factors for asthma exacerbation during pregnancy. The overall risk of adverse obstetrical and perinatal outcomes in women with actively managed asthma during pregnancy is comparable to that in the background population, although the risk of preeclampsia and SGA infants seems to be increased in asthmatic women.

The consensus in the field of asthma and pregnancy is that one-third of women will experience an improvement of their asthma, one-third have no change, and one-third have worsening of their asthma based on subjective assessment (20). However, these changes are not easily predicted, and Schatz et al. (20) have demonstrated that subjective improvement does not predict the course of asthma during pregnancy. Regular monitoring of asthma every four to six weeks during pregnancy is recommended for all pregnant women with asthma (1, 13), as the general paradigm is that the course of asthma during pregnancy is largely unpredicta-

ble (13, 49, 50). The combination of objective and subjective parameters to predict the course of asthma has, as far as we know, not been investigated systematically. We found (study I), that a combination of subjective and objective variables along with the medical history can predict an exacerbation during pregnancy with 98% sensitivity. These results are promising and would allow subgroups of women to be allocated to less intense follow-up, preferably by their general practitioner (GP). Besides making resource allocation more reasonable, such an approach could reduce unnecessary medicalisation of young pregnant women with asthma.

We found a low overall risk of adverse obstetrical and perinatal outcomes in the women followed in the MAP study (study IV). This is in line with a study by Schatz et al. (43) who found that the overall perinatal prognosis for women with actively managed asthma during pregnancy is comparable to that for the non-asthmatic population. Furthermore, we found that enrollment into MAP reduced the number of cases with uncontrolled asthma (study IV), which confirms a recent study suggesting that asthma control rather than exacerbations during pregnancy seem to be related to the risk of adverse perinatal outcomes (51).

In study II, we found that both excessive GWG in first trimester and total GWG are risk factors for exacerbations of asthma during pregnancy. Obesity is associated with persistent low-grade systemic inflammation, and by that obesity is regarded as a pro-inflammatory state (52). Visceral adipose tissue is an important source of cytokine production, whereby adiposity contributes to the pro-inflammatory milieu and is responsible for the formation of low-grade chronic inflammation (52). GWG has also been associated with elevated level of leptin (53, 54) and leptin stimulates the production of inflammatory mediators such as TNF-α and IL-6 from the adipose tissue, which promotes the expression and release of leptin from the adipose tissue, and thereby establish a positive feedback mechanism (52). Excessive GWG during pregnancy can also induce an inflammatory process through insulin resistance (55), and circulating cytokines (i.e., TNF-α concentration) are inversely related to insulin sensitivity (56). Asthma is characterized by chronic airway inflammation, and the level of TNF-α is higher in sputum (57), cells obtained by broncho-alveolar lavage (58) and in bronchial biopsies (59) of subjects with asthma compared to subjects without asthma. Direct administration of TNF-α to the airways of normal volunteers has also induced an increase in the level of bronchial responsiveness (60). The inflammation triggered by excessive GWG mediated by elevated level of TNF-α could possibly explain the increased risk of exacerbations. However, the effect of systemic inflammation on airway inflammation in asthma is debated and only incompletely understood.

Not only does GWG increase the risk of an exacerbation during pregnancy in our study but may also increase the risk of maternal and neonatal complications in women without asthma (61, 62). These findings emphasize the importance of advising also pregnant women with asthma to focus on a healthy weight gain during pregnancy in order to reduce the risk of potential asthma-related pregnancy complications.

We found no association between pre-pregnancy obesity and the risk of asthma exacerbation during pregnancy (study II), which is in accordance with another recent study (51). Even though data from non-pregnant adults indicates that overweight and obesity is associated with worsening of asthma (35), only one study have found the association between obesity and asthma exacerbations during pregnancy (32).

Airway hyperresponsiveness (AHR) is a characteristic feature of asthma, and is measured by direct or indirect bronchial provocation tests (BPTs) (63). A direct BPT, e.g. a methacholine challenge test, causes airway narrowing by acting “directly” on receptors on the bronchial smooth muscle (63). While indirect BPT, e.g. mannitol challenge test, causes airway narrowing “indirectly” by triggering release of a wide variety of mediators from inflammatory cells which act on receptors and cause bronchial smooth muscle contraction (64). In study III we found, that women experiencing an asthma exacerbation during pregnancy were more responsive to mannitol compared to women without exacerbations during pregnancy. This is in line with studies showing that moderate or severe AHR to both direct and indirect BPTs are associated with more frequent symptoms than mild or no AHR (65, 66). Furthermore, increased reactivity to mannitol is a more reliable indicator of loss of disease control than either perceived symptoms or lung function (67). Women who did not experienced an exacerbation during pregnancy (study IV), were less responsive to mannitol. Koskela et al. (68) have demonstrated a reduction in responsiveness to mannitol following ICS treatment, whereas others have found false negative test in subjects treated with ICS (68, 69) . We also found, that AHR to mannitol was associated with sputum neutrophilia, which is in contrast to two other studies (70, 71). However, AHR can occur in the absence of eosinophilic infiltration of the airway mucosa (72, 73), and one study has shown that in the absence of eosinophilic airway inflammation AHR is accompanied by a mast cell infiltration of the airway smooth muscle (72).

We have a unique population as all women in study III were treated with ICS, and the ICS treatment was adjusted if necessary every four weeks during pregnancy based on, among other factors, the level of FENO. FENO is a measure of eosinophilic airway inflammation (74) and FENO-guided management of asthma reduces asthma exacerbations in pregnant women (48). A reduction in eosinophilic airway inflammation due to ICS treatment may have prevented some exacerbations in our study. This indicates that women experiencing an asthma exacerbation, despite close monitoring, may be women with predominantly neutrophilic inflammation.

Limitations

Since 2007, all women referred for delivery at Hvidovre Hospital have received an invitation to be enrolled into the MAP program, but as participation is optional, a risk of selection bias is possible Based on the prevalence of asthma among younger women in Denmark, it is estimated that approximately 2/3 of pregnant women with asthma at Hvidovre Hospital are enrolled in the MAP program: Of the app 300 women referred annually with asthma during pregnancy about 200 fulfill the criteria for inclusion in the MAP cohort. The main reason for exclusion was the visit to the outpatient clinic later than 18 weeks of pregnancy. Furthermore, women enrolled in the MAP cohort are comparable to the background population of pregnant women with regard to age and marital status (study IV).

The frequency of nulliparous among women giving birth at Hvidovre Hospital is 52% (unpublished data) compared to 68% in our cohort. Gesche et al. (75) have also showed that a higher proportion of nulliparous women accept an invitation to participate in a lifestyle interventional trial addressing GWG during pregnancy,

probably reflecting that nulliparous women have more resources to address health issues. Although not formally analyzed, this is supported by our experience of a higher frequency of nulliparous women enrolled into the MAP cohort and the first before 18 weeks of pregnancy.

In our study, severe exacerbations were defined as exacerbation requiring hospital admission, emergency department treatment or/and a rescue course of OCS. The degree of lung function impairment was not part of the exacerbation definition in our study, as the women were not examined in the outpatient clinic by routine at the time of an exacerbation; however, some were seen at unscheduled visit. This is in line with other studies (76, 77).

Mild exacerbations were defined as exacerbations managed by an increase in primarily inhaled therapy, though the definition of mild exacerbation is debatable (18). However, only in study IV our results rely on the definition of mild exacerbation, as we were not able to investigate the severe exacerbations separately due to the modest number of subjects.

A reduction in prevalence of uncontrolled asthma of 80% was observed following enrollment in the MAP study. Furthermore, the majority of exacerbations in our study occurred in first trimester of pregnancy in contrast to other studies (78). The four-weekly consultation with a specialist during pregnancy may have modified the risk of exacerbation as uncontrolled asthma is a well-known risk factor for an exacerbation (1). Women with a low risk of exacerbation during pregnancy i.e. with no history of pre-pregnancy exacerbations, no prescription of controller medication, and clinically stable asthma at the first visit could have experienced an exacerbation if they were followed less intense. We may have reduced the risk of an exacerbation by monthly assessment and adjustment of medication during pregnancy. And the lack of a control group of women allocated to standard care is also a limitation of the study.

We chose to define the control group in the case-control study (Study IV) as the next three deliveries occurring the same day at the department after each of the pregnancies included in the MAP study. These women were identified in a local database by the date and time of delivery, and are very likely to be representative for the background population, but might include women with asthma not responding to the invitation to participate in the MAP program.

Finally, the limitations of the MAP study are the same as other prospective observational cohort studies. Most importantly, observational studies reveal relationship between two types of events that may simply be associated due to coincidence or other common factor. Nonetheless, observational studies are good to generate hypotheses and can give lots of valuable information, which is of outmost importance for designing clinically relevant controlled trials. Even though, we have a large cohort and have demonstrated statistically significant differences, it should be remembered that there is a difference between statistical significant differences and clinically important differences.

Conclusions

The following conclusions can be drawn from the present thesis:

- No history of pre-pregnancy exacerbations, no prescribed controller medication, and clinically stable asthma at the first visit are determinants of pregnancies with a low risk of an asthma exacerbation during pregnancy

- Excessive GWG in first trimester is associated with increased risk of asthma exacerbation during pregnancy. The impact of first trimester GWG on the risk of an exacerbation during pregnancy is dose-dependent with a low risk in women with a GWG less than 5 kg and an increasing risk with every additional kilogram weight gain hereafter.

- A maternal total gestational weight gain of more than 13 kg is significantly associated with increased risk of an asthma exacerbation during pregnancy

- Women experiencing an asthma exacerbation during pregnancy have more pronounced airway hyperresponsiveness

- Non-atopy appears to characterise women at higher risk of an exacerbation of asthma during pregnancy

- The overall risk of adverse obstetrical and perinatal outcomes in women with asthma followed in out-patient clinic, Department of Pulmonary Medicine, during pregnancy is low

- Maternal asthma during pregnancy is associated with an increased risk of SGA infants which increases with increasing asthma severity

- Maternal asthma is a risk factor for mild to moderate pre-eclampsia

Future perspectives

As asthma is the most frequent medical disease in pregnant women and both asthma and exacerbation seems to affect pregnancy outcome, it is important to identify women at increased risk of exacerbations to prevent complications.

It remains to be proven that intense follow-up of asthma during pregnancy is beneficial to all pregnant women with asthma. Future studies should investigate the difference in exacerbation rate in women with intense follow-up compared to standard care in a randomized controlled trial. A possible design could be that women with no previous history of acute exacerbations, no prescribed controller medication, and clinically stable asthma could be allocated to routine follow-up by their GP or to a more intense follow-up program at the pulmonologist. This would allow a proper evaluation of current guidelines on the intense follow-up program.

The evidence is lacking whether an early evaluation of airway inflammation by induction of sputum can predict an exacerbation of asthma during pregnancy. To evaluate the airway inflammation by induction of sputum as early in pregnancy as possible would allow an investigation of potential differences in inflammatory characteristics between women with and without an asthma exacerbation during pregnancy more accurately. Even though we know that airway inflammation is stable over longer term, there is no study showing that the airway inflammation is stable during pregnancy.

Lastly, it would be interesting to investigate if interventions aiming at keeping the GWG in accordance with the recommendation (with diet or exercise), may reduce the risk of asthma exacerbation during pregnancy, and by that prevent potential complications associated with exacerbations of asthma during pregnancy. Such interventions should preferably be implemented in early pregnancy, or even better before pregnancy, as excessive GWG in first trimester is a risk factor for an exacerbation.

Abbreviations

AHR = Airway Hyperresponsiveness
ATS = American Thoracic Society
BMI = Body Mass Index
BPT = Bronchial Provocation Test
ERS = European Respiratory Society

FENO = Fractional Exhaled Nitric Oxide
FEV1 = Forced Expiratory Volume in first second
FVC = Forced Vital Capacity
GINA = Global Initiative for Asthma
GP = General Practitioner
GWG = Gestational Weight Gain
ICS = Inhaled Corticosteroids
LABA = Long Acting β-Agonist
LCL = Lower Confidence Limit
LLN = Lower Limit of Normal
MAP = Management of Asthma during Pregnancy
NHLBI = National Heart Lung, and Blood Institute
NPV = Negative Predictive Value
OCS = Oral Corticosteroids
OR = Odds Ratio
ROC = Receiver Operating Characteristics
RDR = Response-Dose Ratio
SABA = Short Acting β-Agonist
SD = Standard Deviation
SGA = Small for Gestational Age
UCL = Upper Confidence Limit

Summary

Background

Asthma is a serious global health issue and the most prevalent chronic disorder among Danish pregnant women. Exacerbations of asthma during pregnancy have been associated with increased risk of adverse pregnancy and perinatal outcomes, and by that making asthma a potential serious medical condition during pregnancy. Monitoring of asthma every four to six weeks is recommended during pregnancy, although evidence is lacking that following this recommendation will improve pregnancy outcome and, not least, be beneficial for all pregnant women with asthma

Aim

The overall aim of the present thesis was to gain more knowledge of the interaction between asthma and pregnancy. The specific research questions were to identify pregnancies with low risk of an exacerbation during pregnancy, to identify risk factors for an exacerbation during pregnancy, and to compare the adverse pregnancy and perinatal outcomes in women without asthma and women with asthma monitored closely as recommended during pregnancy.

Methods

In study I and II, determinants of pregnancies with low risk of an exacerbation and maternal pregnancy-related risk factors for an exacerbations were investigated in a large prospective cohort study with 1.283 women with asthma. The Management of Asthma during Pregnancy (MAP) was initiated in 2007, and all pregnant women referred to Hvidovre Hospital have since then received an invitation to participate. Women were followed-up every four weeks with assessment of asthma control and adjustment of medication if necessary.

In study III, the potential differences in airway hyperresponsiveness and airway inflammation, in participants (n=50) from the MAP cohort, were investigated in a post-partum examination.

In study IV, the effect of maternal asthma on obstetrical and perinatal outcomes was investigated in a large case-control study, with 938 cases i.e. women with asthma from the MAP cohort, and 2.778 controls i.e. women without asthma.

Results

No history of pre-pregnancy exacerbations, no prescribed controller medication, and clinically stable asthma at the first visit was determinants of pregnancies with a low risk of an asthma exacerbation during pregnancy (study I). Excessive gestational weight gain (GWG) in first trimester was associated with increased risk of an asthma exacerbation during pregnancy; furthermore, the impact of GWG was dose-dependent (Study II).

In study III, women experiencing an asthma exacerbation during pregnancy had more pronounced airway hyperresponsiveness and were more often non-atopic. Finally, in study IV, the overall risk of adverse obstetrical and perinatal outcomes in women with asthma monitored closely during pregnancy was low.

Conclusion

Women with no history of pre-pregnancy exacerbations, no prescribed controller medication, and clinically stable asthma at the first visit have a very low risk of an exacerbation. Furthermore, excessive GWG, airway hyperresponsiveness and being non-atopic are risk factors for exacerbations of asthma during pregnancy. However, the overall risk of adverse obstetrical and perinatal outcomes in women with actively managed asthma during pregnancy is comparable to women without asthma

Reference list

1. Global Initiative for Asthma (GINA). Global strategy for asthma management and prevention www.ginasthma.com [updated 2017].

2. Henriksen L, Simonsen J, Haerskjold A, Linder M, Kieler H, Thomsen SF, et al. Incidence rates of atopic dermatitis, asthma, and allergic rhinoconjunctivitis in Danish and Swedish children. The Journal of allergy and clinical immunology. 2015;136(2):360-6 e2.

3. Traulsen LK, Baelum J, Halling A, Thomsen G, Thilsing T, Sherson D, et al. Risk factors for incident asthma and COPD in a cohort of young adults. The clinical respiratory journal. 2017.

4. Davidsen JR. Drug utilization and asthma control among young Danish adults with asthma. Analyses of trends and determinants. Danish medical journal. 2012;59(8):B4501.

5. Browatzki A, Ulrik CS, Lange P. Prevalence and severity of self-reported asthma in young adults, 1976-2004. The European respiratory journal. 2009;34(5):1046-51.

6. Leynaert B, Sunyer J, Garcia-Esteban R, Svanes C, Jarvis D, Cerveri I, et al. Gender differences in prevalence, diagnosis and incidence of allergic and non-allergic asthma: a population-based cohort. Thorax. 2012;67(7):625-31.

7. Schatz M, Camargo CA, Jr. The relationship of sex to asthma prevalence, health care utilization, and medications in a large managed care organization. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology. 2003;91(6):553-8.

8. Jolving LR, Nielsen J, Kesmodel US, Nielsen RG, Beck-Nielsen SS, Norgard BM. Prevalence of maternal chronic diseases during pregnancy - a nationwide population based study from 1989 to 2013. Acta obstetricia et gynecologica Scandinavica. 2016;95(11):1295-304.

9. Ali Z, Hansen AV, Ulrik CS. Exacerbations of asthma during pregnancy: Impact on pregnancy complications and outcome. Journal of obstetrics and gynaecology : the journal of the Institute of Obstetrics and Gynaecology. 2016;36(4):455-61.

10. McCallister JW, Benninger CG, Frey HA, Phillips GS, Mastronarde JG. Pregnancy related treatment disparities of acute asthma

exacerbations in the emergency department. Respiratory medicine. 2011;105(10):1434-40.
11. Cydulka RK, Emerman CL, Schreiber D, Molander KH, Woodruff PG, Camargo CA, Jr. Acute asthma among pregnant women presenting to the emergency department. American journal of respiratory and critical care medicine. 1999;160(3):887-92.
12. Cossette B, Beauchesne MF, Forget A, Lemiere C, Larivee P, Rey E, et al. Systemic corticosteroids for the treatment of asthma exacerbations during and outside of pregnancy in an acute-care setting. Respiratory medicine. 2014;108(9):1260-7.
13. NAEPP expert panel report. Managing asthma during pregnancy: recommendations for pharmacologic treatment-2004 update. The Journal of allergy and clinical immunology. 2005;115(1):34-46.
14. Bateman ED, Hurd SS, Barnes PJ, Bousquet J, Drazen JM, FitzGerald M, et al. Global strategy for asthma management and prevention: GINA executive summary. The European respiratory journal. 2008;31(1):143-78.
15. Bai TR, Vonk JM, Postma DS, Boezen HM. Severe exacerbations predict excess lung function decline in asthma. The European respiratory journal. 2007;30(3):452-6.
16. Pauwels RA, Lofdahl CG, Postma DS, Tattersfield AE, O'Byrne P, Barnes PJ, et al. Effect of inhaled formoterol and budesonide on exacerbations of asthma. Formoterol and Corticosteroids Establishing Therapy (FACET) International Study Group. The New England journal of medicine. 1997;337(20):1405-11.
17. Fuhlbrigge A, Peden D, Apter AJ, Boushey HA, Camargo CA, Jr., Gern J, et al. Asthma outcomes: exacerbations. The Journal of allergy and clinical immunology. 2012;129(3 Suppl):S34-48.
18. Reddel HK, Taylor DR, Bateman ED, Boulet LP, Boushey HA, Busse WW, et al. An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. American journal of respiratory and critical care medicine. 2009;180(1):59-99.
19. Riley EH, Fuentes-Afflick E, Jackson RA, Escobar GJ, Brawarsky P, Schreiber M, et al. Correlates of prescription drug use during pregnancy. Journal of women's health (2002). 2005;14(5):401-9.
20. Schatz M, Harden K, Forsythe A, Chilingar L, Hoffman C, Sperling W, et al. The course of asthma during pregnancy, post partum, and with successive pregnancies: a prospective analysis. The Journal of allergy and clinical immunology. 1988;81(3):509-17.
21. Apter AJ, Greenberger PA, Patterson R. Outcomes of pregnancy in adolescents with severe asthma. Archives of internal medicine. 1989;149(11):2571-5.
22. Murphy VE, Gibson P, Talbot PI, Clifton VL. Severe asthma exacerbations during pregnancy. Obstetrics and gynecology. 2005;106(5 Pt 1):1046-54.
23. Gluck JC, Gluck P. The effects of pregnancy on asthma: a prospective study. Annals of allergy. 1976;37(3):164-8.
24. Stenius-Aarniala B, Piirila P, Teramo K. Asthma and pregnancy: a prospective study of 198 pregnancies. Thorax. 1988;43(1):12-8.
25. Schatz M, Dombrowski MP, Wise R, Thom EA, Landon M, Mabie W, et al. Asthma morbidity during pregnancy can be predicted by severity classification. The Journal of allergy and clinical immunology. 2003;112(2):283-8.
26. Schatz M, Leibman C. Inhaled corticosteroid use and outcomes in pregnancy. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology. 2005;95(3):234-8.
27. Beckmann CA. A descriptive study of women's perceptions of their asthma during pregnancy. MCN The American journal of maternal child nursing. 2002;27(2):98-102.
28. Nordeng H, Ystrom E, Einarson A. Perception of risk regarding the use of medications and other exposures during pregnancy. European journal of clinical pharmacology. 2010;66(2):207-14.
29. Baarnes CB, Hansen AV, Ulrik CS. Enrolment in an Asthma Management Program during Pregnancy and Adherence with Inhaled Corticosteroids: The 'Management of Asthma during Pregnancy' Program. Respiration; international review of thoracic diseases. 2016;92(1):9-15.
30. Stenius-Aarniala BS, Hedman J, Teramo KA. Acute asthma during pregnancy. Thorax. 1996;51(4):411-4.
31. Lim AS, Stewart K, Abramson MJ, George J. Management of asthma in pregnant women by general practitioners: a cross sectional survey. BMC family practice. 2011;12:121.
32. Hendler I, Schatz M, Momirova V, Wise R, Landon M, Mabie W, et al. Association of obesity with pulmonary and nonpulmonary complications of pregnancy in asthmatic women. Obstetrics and gynecology. 2006;108(1):77-82.
33. Murphy VE, Clifton VL, Gibson PG. The effect of cigarette smoking on asthma control during exacerbations in pregnant women. Thorax. 2010;65(8):739-44.
34. Grarup PA, Janner JH, Ulrik CS. Passive smoking is associated with poor asthma control during pregnancy: a prospective study of 500 pregnancies. PloS one. 2014;9(11):e112435.
35. Schatz M, Zeiger RS, Yang SJ, Chen W, Sajjan S, Allen-Ramey F, et al. Prospective Study on the Relationship of Obesity to Asthma Impairment and Risk. The journal of allergy and clinical immunology In practice. 2015;3(4):560-5 e1.
36. Cnattingius S, Bergstrom R, Lipworth L, Kramer MS. Prepregnancy weight and the risk of adverse pregnancy outcomes. The New England journal of medicine. 1998;338(3):147-52.
37. Cedergren MI. Maternal morbid obesity and the risk of adverse pregnancy outcome. Obstetrics and gynecology. 2004;103(2):219-24.
38. Murphy VE, Namazy JA, Powell H, Schatz M, Chambers C, Attia J, et al. A meta-analysis of adverse perinatal outcomes in women with asthma. BJOG : an international journal of obstetrics and gynaecology. 2011;118(11):1314-23.
39. Wang G, Murphy VE, Namazy J, Powell H, Schatz M, Chambers C, et al. The risk of maternal and placental complications in pregnant women with asthma: a systematic review and meta-analysis. The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet. 2014;27(9):934-42.
40. Bakhireva LN, Schatz M, Jones KL, Chambers CD. Asthma control during pregnancy and the risk of preterm delivery or impaired fetal growth. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology. 2008;101(2):137-43.
41. Firoozi F, Lemiere C, Ducharme FM, Beauchesne MF, Perreault S, Berard A, et al. Effect of maternal moderate to severe asthma on perinatal outcomes. Respiratory medicine. 2010;104(9):1278-87.
42. Namazy JA, Murphy VE, Powell H, Gibson PG, Chambers C, Schatz M. Effects of asthma severity, exacerbations and oral corticosteroids on perinatal outcomes. The European respiratory journal. 2013;41(5):1082-90.

43. Schatz M, Zeiger RS, Hoffman CP, Harden K, Forsythe A, Chilingar L, et al. Perinatal outcomes in the pregnancies of asthmatic women: a prospective controlled analysis. American journal of respiratory and critical care medicine. 1995;151(4):1170-4.
44. Schatz M, Zeiger RS, Hoffman CP. Intrauterine growth is related to gestational pulmonary function in pregnant asthmatic women. Kaiser-Permanente Asthma and Pregnancy Study Group. Chest. 1990;98(2):389-92.
45. Murphy VE, Gibson PG. Asthma in pregnancy. Clinics in chest medicine. 2011;32(1):93-110, ix.
46. Schatz M, Dombrowski MP. Clinical practice. Asthma in pregnancy. The New England journal of medicine. 2009;360(18):1862-9.
47. Kircher S, Schatz M, Long L. Variables affecting asthma course during pregnancy. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology. 2002;89(5):463-6.
48. Powell H, Murphy VE, Taylor DR, Hensley MJ, McCaffery K, Giles W, et al. Management of asthma in pregnancy guided by measurement of fraction of exhaled nitric oxide: a double-blind, randomised controlled trial. Lancet (London, England). 2011;378(9795):983-90.
49. Schatz M. Interrelationships between asthma and pregnancy: a literature review. The Journal of allergy and clinical immunology. 1999;103(2 Pt 2):S330-6.
50. Zairina E, Abramson MJ, McDonald CF, Rochford P, Nolan G, Stewart K, et al. A prospective cohort study of pulmonary function during pregnancy in women with and without asthma. The Journal of asthma : official journal of the Association for the Care of Asthma. 2016;53(2):155-63.
51. Grzeskowiak LE, Smith B, Roy A, Dekker GA, Clifton VL. Patterns, predictors and outcomes of asthma control and exacerbations during pregnancy: a prospective cohort study. ERJ open research. 2016;2(1).
52. Ali Z, Ulrik CS. Obesity and asthma: a coincidence or a causal relationship? A systematic review. Respiratory medicine. 2013;107(9):1287-300.
53. Karakosta P, Georgiou V, Fthenou E, Papadopoulou E, Roumeliotaki T, Margioris A, et al. Maternal weight status, cord blood leptin and fetal growth: a prospective mother-child cohort study (Rhea study). Paediatric and perinatal epidemiology. 2013;27(5):461-71.
54. Ferraro ZM, Qiu Q, Gruslin A, Adamo KB. Excessive gestational weight gain and obesity contribute to altered expression of maternal insulin-like growth factor binding protein-3. International journal of women's health. 2013;5:657-65.
55. Stupin JH, Arabin B. Overweight and Obesity before, during and after Pregnancy: Part 1: Pathophysiology, Molecular Biology and Epigenetic Consequences. Geburtshilfe und Frauenheilkunde. 2014;74(7):639-45.
56. Kirwan JP, Hauguel-De Mouzon S, Lepercq J, Challier JC, Huston-Presley L, Friedman JE, et al. TNF-alpha is a predictor of insulin resistance in human pregnancy. Diabetes. 2002;51(7):2207-13.
57. Obase Y, Shimoda T, Mitsuta K, Matsuo N, Matsuse H, Kohno S. Correlation between airway hyperresponsiveness and airway inflammation in a young adult population: eosinophil, ECP, and cytokine levels in induced sputum. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology. 2001;86(3):304-10.
58. Ying S, Robinson DS, Varney V, Meng Q, Tsicopoulos A, Moqbel R, et al. TNF alpha mRNA expression in allergic inflammation. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 1991;21(6):745-50.
59. Bradding P, Roberts JA, Britten KM, Montefort S, Djukanovic R, Mueller R, et al. Interleukin-4, -5, and -6 and tumor necrosis factor-alpha in normal and asthmatic airways: evidence for the human mast cell as a source of these cytokines. American journal of respiratory cell and molecular biology. 1994;10(5):471-80.
60. Thomas PS. Tumour necrosis factor-alpha: the role of this multifunctional cytokine in asthma. Immunology and cell biology. 2001;79(2):132-40.
61. Hantoushzadeh S, Sheikh M, Bosaghzadeh Z, Ghotbizadeh F, Tarafdari A, Panahi Z, et al. The impact of gestational weight gain in different trimesters of pregnancy on glucose challenge test and gestational diabetes. Postgraduate medical journal. 2016;92(1091):520-4.
62. Liu Z, Ao D, Yang H, Wang Y. Gestational weight gain and risk of gestational diabetes mellitus among Chinese women. Chinese medical journal. 2014;127(7):1255-60.
63. Leuppi JD. Bronchoprovocation tests in asthma: direct versus indirect challenges. Current opinion in pulmonary medicine. 2014;20(1):31-6.
64. Anderson SD. Indirect challenge tests: Airway hyperresponsiveness in asthma: its measurement and clinical significance. Chest. 2010;138(2 Suppl):25S-30S.
65. Peat JK, Salome CM, Sedgwick CS, Kerrebijn J, Woolcock AJ. A prospective study of bronchial hyperresponsiveness and respiratory symptoms in a population of Australian schoolchildren. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 1989;19(3):299-306.
66. Quaedvlieg V, Sele J, Henket M, Louis R. Association between asthma control and bronchial hyperresponsiveness and airways inflammation: a cross-sectional study in daily practice. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2009;39(12):1822-9.
67. Leuppi JD, Salome CM, Jenkins CR, Anderson SD, Xuan W, Marks GB, et al. Predictive markers of asthma exacerbation during stepwise dose reduction of inhaled corticosteroids. American journal of respiratory and critical care medicine. 2001;163(2):406-12.
68. Koskela HO, Hyvarinen L, Brannan JD, Chan HK, Anderson SD. Sensitivity and validity of three bronchial provocation tests to demonstrate the effect of inhaled corticosteroids in asthma. Chest. 2003;124(4):1341-9.
69. Brannan JD, Koskela H, Anderson SD, Chan HK. Budesonide reduces sensitivity and reactivity to inhaled mannitol in asthmatic subjects. Respirology (Carlton, Vic). 2002;7(1):37-44.
70. Porsbjerg C, Brannan JD, Anderson SD, Backer V. Relationship between airway responsiveness to mannitol and to methacholine and markers of airway inflammation, peak flow variability and quality of life in asthma patients. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2008;38(1):43-50.
71. Porsbjerg CM, Gibson PG, Pretto JJ, Salome CM, Brown NJ, Berend N, et al. Relationship between airway pathophysiology and airway inflammation in older asthmatics. Respirology (Carlton, Vic). 2013;18(7):1128-34.
72. Berry M, Morgan A, Shaw DE, Parker D, Green R, Brightling C, et al. Pathological features and inhaled corticosteroid response of

eosinophilic and non-eosinophilic asthma. Thorax. 2007;62(12):1043-9.
73. Wenzel SE, Schwartz LB, Langmack EL, Halliday JL, Trudeau JB, Gibbs RL, et al. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. American journal of respiratory and critical care medicine. 1999;160(3):1001-8.
74. Jatakanon A, Lim S, Kharitonov SA, Chung KF, Barnes PJ. Correlation between exhaled nitric oxide, sputum eosinophils, and methacholine responsiveness in patients with mild asthma. Thorax. 1998;53(2):91-5.
75. Gesche J, Renault K, Norgaard K, Nilas L. Representativeness of participants in a lifestyle intervention study in obese pregnant women - the difference between study participants and non-participants. Obesity facts. 2014;7(6):351-60.
76. Walsh CJ, Zaihra T, Benedetti A, Fugere C, Olivenstein R, Lemiere C, et al. Exacerbation risk in severe asthma is stratified by inflammatory phenotype using longitudinal measures of sputum eosinophils. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2016;46(10):1291-302.
77. Kim JL, Henneberger PK, Lohman S, Olin AC, Dahlman-Hoglund A, Andersson E, et al. Impact of occupational exposures on exacerbation of asthma: a population-based asthma cohort study. BMC pulmonary medicine. 2016;16(1):148.
78. Murphy VE, Clifton VL, Gibson PG. Asthma exacerbations during pregnancy: incidence and association with adverse pregnancy outcomes. Thorax. 2006;61(2):169-76.