

Bronchial asthma and pregnancy. Clinical and cellular immunonological studies

Ph.D. theses

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1. INTRODUCTION

Asthma is one of the most common chronic diseases complicating pregnancy that affects 3.7-8.4% of all pregnancies. The relationship between asthma and pregnancy is bidirectional. Pregnancy has an effect on the course of asthma and asthma influences the outcome of pregnancy. Drug treatment and management of asthmatic pregnant women represent a special challenge for pulmonologists.

The fetus is a semi-allograft for the maternal immune system. The physiological tolerogenic maternal immune reaction is essential for successful pregnancy. The pregnancy-induced immunological changes are systemic. However little is known about immunological changes characterizing asthmatic pregnancy.

Measurement of fractionated exhaled nitric oxide (FeNO) is a useful tool for monitoring airway eosinophilic inflammation in asthma. However, until now its value has not been examined in asthmatic pregnant patients.

2. OBJECTIVES

Exhaled fraction of nitric oxide (FeNO) in healthy and asthmatic pregnancy

In our clinical study we aimed to answer the following questions 1) if the currently available technique for FeNO measurement is reproducible in pregnant women, 2) whether pregnancy itself influences FeNO values in pregnant subjects, 3) whether FeNO values are related to asthma control in pregnant asthmatic patients.

Cellular immunological studies:

Study 1. Circulating T lymphocyte profiles in asthmatic pregnancy

Study 2. Effector and regulatory T lymphocytes in asthmatic pregnancy

In our cellular immunological studies we aimed to assess 1) if signs of pregnancy-induced immune tolerance can be detected in healthy pregnancy, 2) if maternal asthma influences pregnancy-induced immune tolerance, 3) if there is any correlation between lymphocyte profiles and neonatal birth weight in asthmatic pregnancy?

In order to answer the above questions we aimed to determine the distribution of various T lymphocytes (naive T cells, memory T lymphocytes, natural killer T cells (NKT), regulatory T lymphocytes (Treg), T helper 1 (Th₁) and T helper 2 (Th₂) cells) and natural killer (NK) cells in the peripheral blood of asthmatic pregnant (AP) and asthmatic non-pregnant (ANP) patients, together with healthy pregnant (HP) and healthy non-pregnant (HNP) women of same age.

3. METHODS

Subjects

A total of 102 women (35 healthy non-pregnant and 27 healthy pregnant women, 20 asthmatic non-pregnant and 20 asthmatic pregnant patients) were enrolled in the FeNO measurement cross-sectional study (Table I.). Twenty one AP, 13 HP, 10 HNP and 12 ANP women were included in Study 1 (Table II/A.), while 61 AP, 33 HP, 15 HNP and 62 ANP women of reproductive age were enrolled in Study 2 (Table II/B.). All asthmatic patients had mild to moderate persistent disease. The severity of asthma and the antiasthmatic treatment were comparable in the two asthmatic groups in both studies. Pregnant subjects were recruited when attending their

scheduled visit at the 1st Department of Gynecology and Obstetrics (Semmelweis University, Budapest). The obstetric data (gestational age, birth weight) came from their medical reports. Exclusion criteria were smoking and any other chronic disease except for allergic rhinitis.

Measurement of asthma control

Asthma control was assessed by the Asthma Control Test (ACT). Results are given in mean± standard deviation (SD).

Lung function

Forced expiratory volume in 1 second (FEV₁) was measured by means of electronic spirometer (PDD-301/s, Piston, Budapest, Hungary) according to the ATS guidelines. Results are given in mean±SD.

Blood gas analyses

Arterialized blood gas [oxygen (pO₂) and carbon dioxide partial pressure (pCO₂) and pH] was analyzed (Stat Profile pHox Basic, Nova Biomedical, Austria) in all asthmatic patients.

Measurement of FeNO

All participants underwent the measurement of FE_{NO} using NIOX MINO[®] Airway Inflammation Monitor (NIOX MINO[®]; Aerocrine AB, Solana, Sweden). Two technically adequate breath maneuvers were performed. There were 3 minutes in between the two measurements. Data are expressed as median and range [median (min, max)].

Analysis lymphocyte subpopulations with multicolor flow cytometry

In Study 1. peripheral venous blood was collected by venipuncture, using the Becton Dickinson (BD, San Jose, CA, USA) Vacutainer[®] Heparin Tubes. Immunophenotypic analysis was performed on the day of sample

collection. All antibodies (CD3, CD4, CD8, CD19, CD25, CD28, CD71, CD 95, CD11b, HLADR) used for the characterization of different cell populations were manufactured by BD Biosciences Pharmingen. The „Direct Immunofluorescence Staining of Cells Using a Lyse/Wash Procedure” protocol of BD Biosciences was used for staining the cell surface antigens. Measurements were carried out using a FACSCalibur flow cytometer (BD). The absolute numbers of cells and their subsets in clinical samples were accomplished using dual-platform counting technologies, which couples the percentage of positive cell subsets counted by flow cytometry and the absolute cell count measured by automated hematology analyzer (Cell-Dyne 3200, Abbott).

In Study 2. multicolor flowcytometry was made from six ml lithium-heparin anticoagulated blood. CXCR3 was assessed as a Th1-associated chemokine receptor, and CCR4 as a Th2-associated chemokine receptor. In addition, naive and memory CD4⁺ cells (CD45RA⁺ and CD45RO⁺, respectively), and the prevalence of NK, NKT, and iNKT cells (CD3⁻CD161⁺, CD3⁺CD161⁺ and CD3⁺6b11⁺, respectively) were also identified. Tregs were defined as intracellular FoxP3⁺ cells among the population of CD4⁺CD25⁺ cells.

4. RESULTS

FeNO in healthy and asthmatic pregnancy

Clinical data

The level of asthma control was similar in pregnant and non-pregnant asthmatics, as there was no significant difference in Asthma Control Test total scores of the two groups (20.78±2.96 vs. 19.17±3.1, p=0.17) (Table I). The daily dose of the inhaled corticosteroid (ICS) was same in the two

asthmatic groups (Table I). Clinical characteristics of the subjects are shown in Table I.

Table I. Clinical characteristics of enrolled subjects. $p>0,05$ for all comparisons.

	HNP	HP	ANP	AP
number	35	27	20	20
Age*(years)	27±2	29±3	31±5	28±4
Gestation age* (weeks)	-	26±8	-	27±7
FEV ₁ *(% of predicted)	-	-	85±10	85±7
ACT total score	-	-	19.17±3.1	20.78±2.96
ICS daily dose (µg) [§]	-	-	775 (200-1000)	675 (250-1500)

*Data are given as mean ± SD; [§]ICS daily dose (beclometason-dipropionate CFC aerosol equivalent) is given as mean (min-max). $p>0,05$ for all comparisons.

Data of FeNO

There was no statistically significant difference between the first and the second FeNO measurement in pregnant subjects, regardless of the value of FeNO (Figure 1.). FeNO was significantly higher in AP group compared to HP group [28 (10, 56) vs. 16 (8, 31) ppb, $p<0.05$], while HP and HNP women had similar values [16 (8, 31) vs. 16 (9, 35) ppb $p>0.05$]. The FeNO in AP group was as high as in ANP group (Figure 2.). Furthermore, a significant negative correlation between FeNO levels and ACT total scores was detected in AP group (Figure 3.).

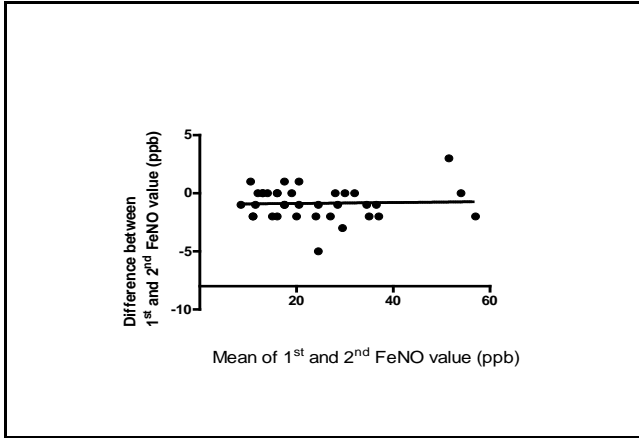


Figure 1. Bland-Altman plot of two measurements in asthmatic pregnant (n=20) and healthy pregnant (n=20) subjects.

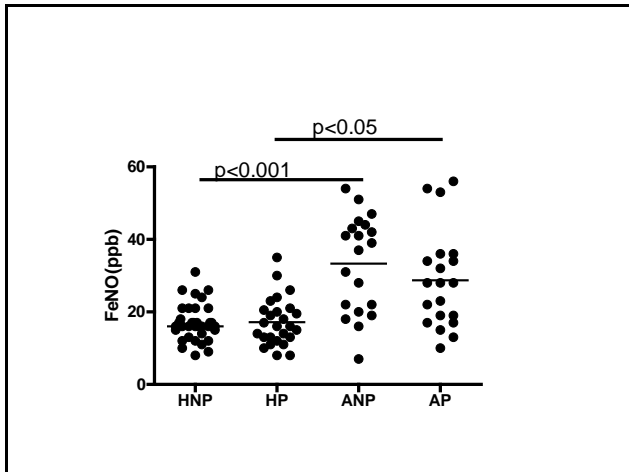


Figure 2. FeNO in n=35 healthy non-pregnant (HNP), n=27 healthy pregnant (HP), n=20 asthmatic non-pregnant (ANP) and n=20 asthmatic pregnant (AP) women.

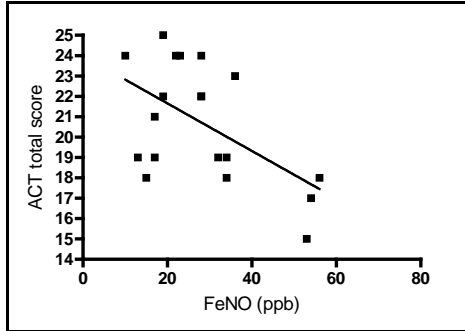


Figure 3. Significant negative correlation between FeNO and Asthma Control Test total scores in asthmatic pregnant patients (FeNO vs. ACT, $p < 0.05$)

Cellular immunological studies

Clinical data

Demographic data, the severity and the control of asthma were similar in pregnant and non-pregnant asthmatic patients (Table II.). Antiasthmatic maintenance treatment was also similar in two asthmatic groups [in Study 1. 9 AP and 11 ANP received ICS in the same dose (587 ± 74 vs. 781 ± 120 , $p > 0.05$); in Study 2. 33 AP and 37 ANP received ICS, the median of the daily dose of ICS was $400 \mu\text{g}$ in both asthmatic group].

Table II. Clinical data of subjects in the first (A) and in the second (B) cellular immunological study.

A.

	HNP	ANP	HP	AP
N	10	12	13	21
Age (year)	29.0±1.6	31.4±1.7 ^{ns}	29.6±1.1 ^{ns}	31.7±1.0 ^{ns}
Gestational age (week) at examination	-	-	26.8±2.0	26.4±1.7 ^{ns}
Distribution of gestational age (1./2./3. trimester)	-	-	0/7/6	0/11/10 ^{ns}
FEV ₁ (% of predicted)	-	86.00 ± 5.59	-	87.62 ± 3.24 ^{ns}

B.

	HNP	ANP	HP	AP
N	15	62	33	61
Age (year)	35(29-39)	31.5(27.8-40.5) ^{ns}	33.0 (30-34.5) ^{ns}	30(28-33.5) ^{ns}
Gestational age (week) at examination	-	-	25 (14.5-34)	24 (14.5-34) ^{ns}
Distribution of gestational age (1./2./3. trimester)	-	-	8/9/16	13/24/24 ^{ns}
FEV ₁ (% of predicted)	-	89.0 (78.8-99.0)	-	91.0 (85.0-97.0) ^{ns}
ACT total score	-	19 (17-22)	-	20.5 (18-24) ^{ns}

Data are given as mean ± SEM in Table A and as median (quartiles) in Table B. n.s.-not significant

Circulating lymphocyte subpopulations

Activated pools of lymphocytes (CD25⁺ and CD95⁺ T cells, CD25⁺, CD28⁺ and CD54⁺ CD4⁺ cells, CD54⁺ and CD11b⁺ CD8⁺ lymphocytes) were elevated in ANP vs. HNP subjects. Lower absolute number of CD3⁺ cells in AP than in ANP patients may reflect the systemic immunotolerogenic effect of pregnancy on asthmatic inflammatory responses. Furthermore, asthma related lymphocyte activation was also missing in asthmatic pregnant. Significant negative correlation observed between CD3⁺CD8⁺, CD3⁺HLADR⁺, CD8⁺CD28⁺ prevalence and FEV₁ values (all p<0.05), as well as between CD3⁺HLADR⁺, CD8⁺CD28⁺ cells and airway resistance (all p<0.05) in AP group suggested a relationship between systemic inflammation and clinical control of asthma. Direct positive correlation (p<0.01) was revealed between the CD95⁺ T cell count and birth weight of newborn in HP group, but no such relationship was demonstrated in AP patients.

The mean gestation age at delivery was similar in the two pregnant groups (38.9±0.3 vs. 39.1±0.3, AP vs. HP, p>0.05), however the birth weight of newborns was lower in AP than in HP patients (3224±145 vs. 3332±93, p<0.05).

Regulatory and effector T lymphocytes were assessed in Study 2. Treg prevalence was higher in HP than in HNP group (Figure 4.) and there was a significant positive correlation between peripheral Treg cell prevalence and the birth weight of newborns in healthy pregnancy (Figure 5.). In AP patients the physiological increase of circulating Treg cells observed in HP was diminished, suggesting altered maternal immunotolerance, together with the lack of correlation between peripheral Treg prevalence and the birth weight of newborn. In pregnancies complicated with asthma, the expected elevation of Treg prevalence in the second and third trimester was

not observed (Figure 6.). Still, in AP patients significantly higher naive and lower memory CD4⁺ T cell prevalence were detected than in ANP group, and the prevalence of NK cells was also less in pregnant than in non-pregnant asthmatics. Our findings indicated no statistically difference between the four groups considering peripheral Th₁ (CXCR3 expressing CD4⁺ T cells) and Th₂ (CCR4 expressing CD4⁺ T lymphocytes).

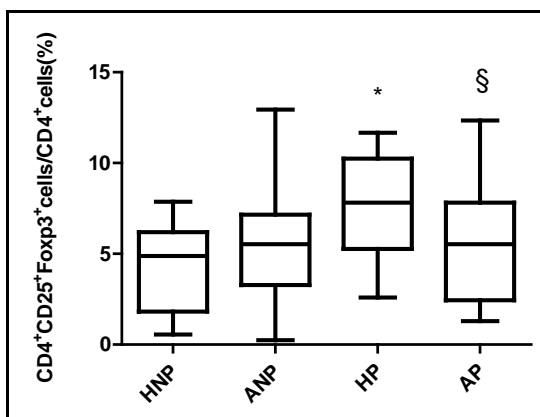


Figure 4. Prevalence of the Treg (CD4⁺CD25⁺Foxp3⁺) cells in the four groups. Box-Whisker plot. $p < 0.05$ vs *HNP, $p < 0.05$ vs §HP with Kruskal-Wallis and Dunn's post hoc test.

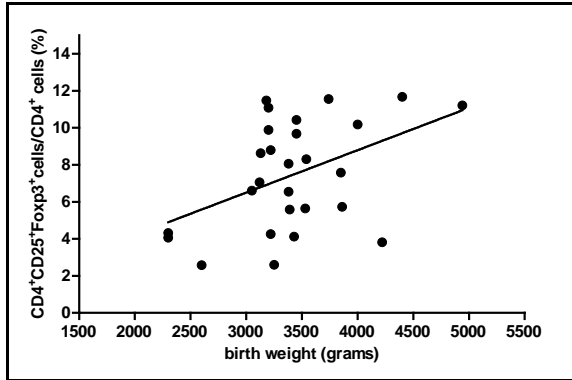


Figure 5. Positive correlation between Treg cell prevalence and birth weight of newborn in healthy pregnancy (n=27, r=0.44, p=0.02).

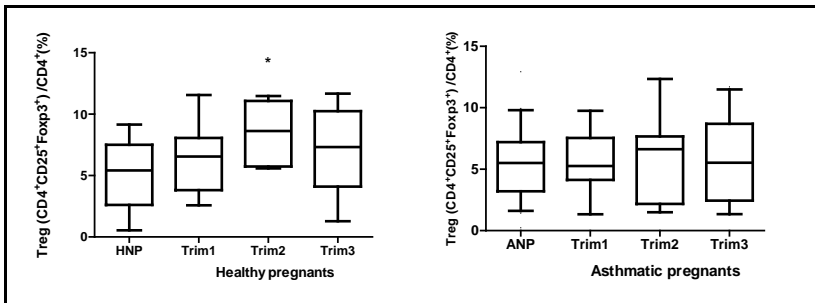


Figure 6. Changes in peripheral Treg ($CD4^+CD25^+Foxp3^+$) cell population sizes during healthy and asthmatic pregnancy. Box and whisker plot showing $CD25^+Foxp3^+CD4^+$ Treg population as a percentage of total $CD4^+$ cells (median, minimum, and maximum ranges) for each trimester (Trim). (p=0.47 Trim 1 vs. healthy non-pregnant (HNP); *p=0.025 Trim 2 vs. HNP; p=0.14 Trim 3 vs. Trim 2).

5. CONCLUSIONS

Measurement of FeNO is a useful tool for monitoring airway eosinophilic inflammation in asthma. Although the placental nitric oxide production increases during pregnancy, according to our data FeNO remains unchanged in healthy pregnancy. The method of FeNO measurement proved to be reproducible in pregnant patients. In AP patients FeNO level increases similarly to ANP patients' data and related to asthma control. Thus, FeNO may represent an adequate method for monitoring asthmatic airway inflammation during pregnancy complicated with asthma.

In the cellular immunological studies we examined the prevalence of various lymphocyte populations (CD4⁺ and CD8⁺ T cells, Treg and NK cells) in pregnant and age-matched non-pregnant women suffering from asthma of similar level of control. Age-matched healthy pregnant and healthy non-pregnant women were included as control subjects. Various signs of asthmatic immunologic activity detected in asthmatic non-pregnant patients were reduced in well controlled asthmatic pregnant patients who were also free from obstetric complications. Increased Treg number detected in healthy pregnancy reflects the immunotolerogenic effect of pregnancy. Blunted physiologic elevation of Treg number in asthmatic pregnant together with diminished correlation between Treg prevalence and birth weight of neonates indicate immunological effect of asthma on pregnancy.

Our results suggest pregnancy induced attenuation of immunological activity in asthma, but also confirm that even mild, well-controlled asthma interferes with the full expression of pregnancy-associated immunotolerance in asthmatic pregnancy.

6. LIST OF PUBLICATION

Publications in English in related to the dissertation:

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