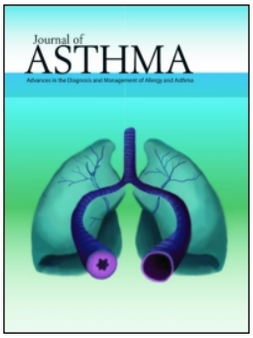

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Circulating periostin level in asthmatic pregnancy

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Abstract

Objective: Asthma often complicates pregnancy and represents a risk for complications. Periostin is considered as a biomarker of asthma; however, as it also plays a role in normal gestation, pregnancy may influence circulating periostin levels. This is the first study assessing periostin in asthmatic pregnancy.

Methods: Plasma periostin levels were investigated in asthma (asthmatic non-pregnant, ANP; N = 19) and asthmatic pregnancy (AP; N = 14), compared to healthy non-pregnant controls (HNP; N = 12) and healthy pregnant women (HP; N = 17). The relationship between periostin levels and asthma control determinants was also evaluated. The diagnostic efficacy of periostin to detect uncontrolled asthma was analyzed using ROC analysis.

Results: Plasma periostin levels were similar in the HNP and ANP (55.68 [37.21-67.20] vs. 45.25 [32.67-64.55], $p > 0.05$), and elevated in the HP (68.81 [57.34-98.84] ng/mL, $p = 0.02$ vs. HNP) and AP groups (54.02 [44.30-74.94] ng/mL, $p = 0.0346$ vs. ANP). Periostin levels of the two pregnant groups were similar ($p > 0.05$). In AP women periostin correlated negatively with FEV_1 ($r = -0.5516$) and positively with Raw ($r = 0.5535$; both $p < 0.05$).

Conclusions: Pregnancy itself increases circulating periostin levels and this elevation is detectable in asthmatic pregnancy as well. Although periostin correlates with lung function in asthmatic pregnancy, periostin as a biomarker has to be handled with caution in pregnant patients due to the influence of pregnancy on its plasma level.

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Introduction

Asthma is a chronic inflammatory disease of the airways characterized by variable and recurrent symptoms, local inflammation, reversible airflow obstruction, and bronchospasm [1]. It has a high burden of morbidity especially if not controlled which occurs in up to 50% of cases worldwide [1]. Asthma is a heterogeneous disease with several phenotypes. Easily obtainable blood biomarkers related to uncontrolled disease would help to identify patients at risk and to phenotype patients favoring a specific treatment which can help to optimize disease outcome; however optimal blood biomarkers are still missing.

At the same time, asthma is one of the most common chronic diseases complicating pregnancy, occurring in 8-12% of all gestations [2]. It represents a risk for potentially serious maternal and fetal morbidities, including preterm delivery, gestational hypertension, preeclampsia, low birth weight, and neonatal mortality [3, 4]. On the other hand pregnancy may also influence asthma control with the deterioration of symptoms in one-third of all asthmatic pregnant women [5]. Notably, if the disease is well controlled the risks of poor pregnancy outcomes decrease [6], therefore, maintaining optimal control in this period is crucial. Hence in asthmatic pregnancy it would be especially important to have clinically usable biomarkers that would indicate the loss of asthma control and would thus help to identify asthmatic pregnant women with elevated risk of pregnancy complications. Pregnancy itself can influence spirometry results [7], and this measurement cannot be performed in all cases because it requires vigorous breathing maneuver. Furthermore, the available techniques used to determine specific asthma phenotypes (e.g. induced sputum) are semi-invasive hence cannot be used in pregnancy. Fractional exhaled nitric

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oxide (FE_{NO}) measurement is a promising non-invasive method which can be utilized also in pregnancy [8], moreover, asthma treatment based on FE_{NO} during pregnancy was able to decrease the occurrence of exacerbations, together with improving life quality and decreasing neonatal hospitalizations compared with traditional asthma management [9]. However, a more recent longitudinal study detected large intraindividual variability in asthmatic pregnant women independently of asthma control [10]. Furthermore, perception of asthma control is subjective and can vary from patient to patient. Therefore circulating, non-invasively obtainable biomarkers related to disease control or lung function would largely support clinical decision-making in this population. Previously two promising blood biomarkers, suPAR and hyaluronic acid were investigated, that were both proven to be usable rather in asthma alone than in asthmatic pregnancy [11, 12].

In the recent years, periostin has emerged as a valuable biomarker in asthma, primarily in its T helper 2 (Th2)-induced, eosinophil-predominant phenotype [13]. Periostin is an extracellular matrix protein, which is expressed at low levels in connective tissue and plays a role in generation of collagen. Its expression is largely increasing in the presence of tissue injury or inflammation, contributing actively to reparatory processes and wound healing [14]. Periostin is also involved in Th2-type allergic inflammation [15], as it was demonstrated in atopic dermatitis [16] and eosinophilic esophagitis [17]. Periostin is among the most highly differentially expressed genes of airway epithelium in asthma [18], and expression of this gene correlates with subepithelial fibrosis [19]. Its contribution to airway fibrosis and remodeling is reflected also in the clinical setting, as increased periostin level at enrollment was associated with a decline in forced expiratory volume in 1 second (FEV_1) of 30 mL or greater per year in an 8-year long

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study [20]. Periostin also facilitates eosinophil infiltration [17], and was shown to be associated with airway eosinophilia in a study examining severe asthmatics on high-doses steroid treatment [21]; moreover it was proven to be the best single predictor of airway eosinophilia [21]. Pathomechanism-based targeted therapy is increasingly important in optimal personalized treatment of asthma. In severe asthma periostin already may influence therapeutic decisions since it can help to choose patients who will respond to anti-interleukin-(IL-)13 treatment [22] or anti IL-5 therapy [23]. Serum periostin levels also correlated with fractional exhaled nitric oxide (FE_{NO}) and with airway hyperresponsiveness to methacholine and mannitol in asthmatic children [24]. More importantly, high serum periostin in patients with high FE_{NO} was a risk for subsequent asthma exacerbations despite taking high-dose ICS, independent of known risks including low FEV_1 [25]. Importantly, circulating periostin levels seemed to be stable within subjects at 3 measurements in a 5-week period [21]. However, biomarkers used in the non-pregnant state are not always applicable in the same way in pregnant women and often require altered reference ranges, as showed e.g. with D-dimer and fibrinogen [26], the erythrocyte sedimentation rate [27] white blood cell counts [28] or C-reactive protein (CRP) levels [29]. Circulating periostin level may be altered by gestation itself as it has a role in embryo implantation and in maintaining normal pregnancy. It induces endometrial decidualization and promotes migration of trophoblastic cells [30, 31]. It is also required for normal embryogenesis [32, 33, 34, 35]. Lower periostin levels were detected in decidua, trophoblastic tissues, and sera in spontaneous abortion compared to healthy pregnant women undergoing voluntary pregnancy termination [36]. On the other hand, serum periostin concentration was further elevated in patients with preeclampsia compared with normotensive pregnant women [37]. However,

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according to our knowledge, there are no data about periostin level or its relationship to asthma symptoms in asthmatic pregnancy.

As circulating periostin level may be influenced by gestation, its usefulness as a biomarker may be altered in pregnant patients. The present study investigated circulating periostin level in pregnant and non-pregnant subjects with or without asthma, with the main aim to evaluate whether pregnancy influences plasma periostin levels in asthmatic patients. The relationship between periostin levels and asthma control determinants was also evaluated. In order to have a more complete view on the inflammatory state of patients, circulating CRP and interleukin (IL)-6 levels, together with peripheral eosinophil cell percentages were measured as well.

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Methods

Ethics Statement

Written informed consent was obtained from the subjects, and our study was reviewed and approved by an independent ethical committee of the institution (Institutional and Regional Research Ethics Committee; 7828-4/2014). The study adhered to the tenets of the most recent revision of the Declaration of Helsinki.

Study participants

The study had a cross-sectional design. 12 healthy non-pregnant (HNP) and 17 healthy pregnant (HP) women, and19 asthmatic non-pregnant (ANP) and 14 asthmatic pregnant (AP) women were enrolled. Asthmatic patients were assessed at their regular visit at the outpatient clinic. They had persistent disease and asthma had been diagnosed according to the current guidelines

[1]. Exclusion criteria were current smoking or more than 5 pack years of smoking history in order to exclude presence of COPD, any other chronic disease (except for allergic rhinitis), acute infection within four weeks of measurement, fetal infection and multi-fetal gestation. Patients were asked not to use their medication 12 hours before visits. Healthy pregnant subjects were recruited when attending their scheduled visit. Healthy non-pregnant controls were volunteers and had a negative history and negative status upon detailed physical and routine laboratory examination.

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Measurement of plasma periostin and other circulating markers

Plasma periostin concentrations were measured with the Human periostin/osteoblast specific factor 2 (POSTN) ELISA Kit (MBS705827; MyBioSource, Inc., San Diego, California, USA). Plasma was isolated from EDTA anticoagulated blood samples between 3-8°C within 30 minutes and stored at −80°C until measurement as recommended by the manufacturer.

CRP, IL-6 and eosinophil percentages were measured as routine laboratory tests using commercially available reagents. CRP values below the level of detection (1 mg/L) were regarded as 1 mg/L. IL-6 values below the level of detection (1.5 pg/mL) were regarded as 1.5 pg/mL.

Lung function measurements and asthma control evaluation

Lung function was measured by means of electronic spirometer (PDD-301/s, Piston, Budapest, Hungary) according to the American Thoracic Society (ATS) guidelines [38]. Three technically acceptable maneuvers were performed and the best was used. Forced expiratory volume in one

second (FEV_1), peak expiratory flow rate (PEF), airway resistance (R_{aw}) and forced expiratory flow at 25--75% of forced vital capacity ($FEF_{25-75\%}$) were recorded. Asthma control was assessed using the Asthma Control Test (ACT) recommended by the current asthma guideline [1].

Statistics

Data distribution was analyzed by D'Agostino-Pearson normality test. Comparisons between the study groups were made with Kruskal-Wallis and Dunn's post hoc multiple comparison tests. Correlation analyses were performed using Spearman's test due to non-normal distribution of data. Area Under Curve (AUC) values of Receiver Operating Characteristics (ROC) curves were calculated using standard methods and data are presented as AUC ROC (95% CI). p values < 0.05 were considered significant. Sample size of the study was calculated to achieve an 80% power to detect 50% (effect size of 0.5) difference among the 4 groups regarding circulating periostin levels.

Statistics was calculated using the Statistica software (version 11, StatSoft, Inc, Tulsa, OK, USA) and Graph Pad Prism software 5 (GraphPad Software, La Jolla, CA, USA). Data are expressed as median [interquartile range].

Results

Clinical characteristics

Clinical data and inflammatory parameters of the four study groups are summarized in Table 1. The median age of participants was comparable between study groups (p > 0.05). Gestational age at blood sampling did not differ between the AP and HP groups (p > 0.05). Although the median

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of newborns' birth weight seemed somewhat lower in the asthmatic pregnant group, there was no significant difference between either gestational age at delivery ($p = 0.43$) or fetal birth weight ($p = 0.19$) in the two pregnant groups.

No difference was detected in the control of asthma or in the prescribed daily dose of inhaled corticosteroids (ICS) between the ANP and AP groups (Table 1). However, forced vital capacity (FVC; 95.5±10.3 vs. 108.3±11.6% of predicted) and peak expiratory flow (PEF; 77.9±15.8 vs. 90.9±13.9% of predicted) were lower in the pregnant than in the non-pregnant patients; the other lung function parameters did not differ. 13 non-pregnant and 8 pregnant asthmatic patients received ICS treatment, while 6 patients were steroid-naïve in both asthmatic groups. Steroid-naïve and steroid-treated patients differed in none of the investigated parameters (such as spirometry results, ACT total scores, inflammatory parameters, peripheral eosinophil cell percentage). Mean ACT total score of 21.65 (±3.39) in the ANP and 20.18 (±4.12) in the AP group showed acceptable and similar level of disease control of the patients in both groups.

Comparison of circulating marker levels among the four groups

Treated and in most cases well-controlled asthma itself did not influence peripheral level of periostin, as it was similar in the HNP and ANP groups (55.68 [37.21-67.20] vs. 45.25 [32.67-64.55], $p > 0.05$; Figure 1 A, Table 1). On the other hand, healthy pregnancy was associated with a marked elevation in periostin level (68.81 [57.34-98.84] ng/mL, $p = 0.02$ vs. HNP; Figure 1 A, Table 1). Similarly, this pregnancy-specific elevation was detectable also in the AP group (54.02 [44.30-74.94] ng/mL, $p = 0.03$ vs. ANP) compared to the ANP group. There was no difference between the periostin levels of the two pregnant groups ($p > 0.05$; Figure 1 A, Table 1).

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Peripheral level of CRP was somewhat higher in the ANP and AP groups compared to the HNP controls (4.0 [3.0-5.5] and 6.0 [3.0-6.5] vs. 3.0 [1.3-3.0] mg/L, respectively, $p < 0.001$; Figure 1 B, Table 1); the two asthmatic groups did not differ. CRP was not measured in the HP group.

We measured peripheral IL-6 levels only in the two asthmatic groups with the result that it was lower in the AP group compared with the ANP one (1.93 [1.50-3.34] vs. 4.21 [1.89-6.95] pg/mL, $p = 0.04$; Figure 1 C, Table 1).

Peripheral percentage of eosinophil granulocytes was higher in the ANP and AP groups compared to the HP group (2.51 [2.09-4.30] and 2.26 [1.37-4.16] vs. 0.88 [0.50-1.00] %, respectively, $p < 0.001$) but not to the HNP one (1.55 [1.26-2.02] %, $p > 0.05$; Figure 1 D, Table 1).

Peripheral periostin concentration was not related to CRP or IL-6 levels in any study group; however, it showed a trend towards a correlation with peripheral eosinophil percentage in AP women ($r = 0.47$, $p = 0.09$). Furthermore, periostin tended to be higher in the AP participants who showed peripheral eosinophilia (as defined by >3% of peripheral white blood cells) compared to ones who did not show eosinophilia but the difference reached only the level of a trend (51.71 [41.45-69.54], $n = 9$ vs. 82.39 [49.76-87.24] ng/mL, $n = 5$, $p = 0.09$). In the ANP group, peripheral eosinophil percentage correlated with IL-6 levels ($r = 0.53$; $p = 0.03$).

Relationship of inflammatory markers to asthma control determinants

Circulating peripheral periostin level was significantly related to lung function in the AP group: it correlated negatively with forced expiratory volume in 1 second (FEV_1; $r = -0.55$; Figure 2 A),

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and positively with airway resistance (R_{aw}; r = 0.55; Figure 2 B; both $p < 0.05$), while in the case of FVC the negative association reached only the level of a trend (r = -0.51, p = 0.06). On the other hand, all of these correlations were missing in the ANP group. Periostin levels were not related to ACT total scores or the dose of the prescribed ICS treatment either in the ANP or in the AP group.

Peripheral eosinophil percentage correlated negatively with FEV_1 in the ANP group (r = -0.53; Figure 3; p = 0.03). However in the AP group peripheral eosinophil percentage showed only a trend towards a negative correlation with FEV_1 (r = -0.52, p = 0.05) and with PEF values (r = -0.49, p = 0.09).

In order to evaluate the efficacy of periostin measurement in the detection of uncontrolled asthma, ROC analyses of periostin data were performed in subgroups of AP and ANP patients with PEF above and below 80% and ACT total score above and below 20, as the current GINA guideline suggests these cut-off values to differentiate between optimal and suboptimal asthma control. None of these investigations yielded significant results. ROC analyses of CRP and IL-6 values were performed also without significant results in any group.

Relationship of inflammatory and functional parameters to obstetrical data

Periostin concentrations were not related to gestational age at sampling either in the AP or in the HP group ($p > 0.05$). There was also no difference between periostin values of women in early pregnancy (first and second trimester) and women in late pregnancy (third trimester) neither in the AP nor in the HP group ($p > 0.05$).

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We could not detect any association between periostin, CRP or IL-6 levels and obstetrical data such as gestational age at delivery and fetal birth weight.

Discussion

Periostin recently became a promising biomarker of Th2-driven airway inflammation and airway eosinophilia in asthma; however, its circulating level has not been examined in asthmatic pregnancy yet. In our study, we measured the peripheral levels of periostin in healthy and asthmatic pregnancy together with healthy and asthmatic non-pregnant patients. We demonstrated a marked increase in its level in pregnancy independently of concomitant asthma, as circulating periostin level was increased both in healthy and asthmatic pregnant, compared to healthy and asthmatic non-pregnant women, respectively. Unexpectedly, similar periostin levels were detected in mostly treated, controlled asthmatic non-pregnant patients and healthy controls; similarly, treated asthmatic and healthy pregnant women had similar periostin levels as well. On the other hand, in asthmatic pregnancy circulating periostin level correlated with worse lung function parameters.

Circulating and tissue periostin levels change during pregnancy but its precise role is not fully elucidated in healthy or pathologic pregnancies. Circulating periostin level was shown to be increased in healthy pregnancy compared with non-pregnant volunteers [36, 37]. In line with this, we measured higher plasma levels in the HP group than in the HNP control group. In AP we found that the pregnancy induced elevation of circulating periostin level was present (compared to non-pregnant patients) suggesting that asthmatic processes do not interfere with the role of periostin in normal gestation. However, due to this pregnancy induced elevation, the value of

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periostin as an asthma biomarker during pregnancy may be compromised. Moreover, potentially altered cut-off values are warranted compared to non-pregnant patients. In line with earlier results [37], periostin concentrations did not show any correlation with birth weight of the infants either in HP or in AP group in our study.

Maintaining optimal disease control is a crucial need in asthmatic pregnancy, however both the value and the safety of spirometry may be challenged. Therefore, measurement of validated non-invasive biomarkers such as periostin may represent an aid in risk assessment and asthma control evaluation in this problematic patient group. However, as periostin plays an important role in normal gestation and its level changes both in healthy and complicated pregnancies, its value as an asthma biomarker may be challenged during asthmatic pregnancy. Our study was the first to detect higher periostin levels in asthmatic gestation compared to non-pregnant patients. Furthermore, according to our data, in asthmatic pregnancy periostin concentrations were inversely associated with $FEV_{1,}$ and positively with R_{aw}; these correlations were absent in the ANP group. The explanation of this discrepancy between the asthmatic groups may be the somewhat differing clinical state of the patients, as PEF and FVC values (expressed as percentage of predicted) were lower in the pregnant than in the non-pregnant patients in our study. Notably, according to earlier results, these two lung function parameters are expected to increase in healthy pregnant women compared to the non-pregnant state; hence in our pregnant asthmatic patients these lung function results have to be regarded as actually worse than they would seem to be in non-pregnant conditions [7] and thus the association between periostin and worse lung function may be more obvious in this group. Optimal lung function and well-

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controlled clinical state could mask the associations between periostin and lung function parameters in the ANP group.

In our study we could not confirm the previous data [20, 39] as we did not detect higher peripheral periostin levels in bronchial asthma (without pregnancy) than in healthy controls. Of note, normal circulating levels of periostin or cut-off values in asthma are not defined yet and different values were considered as elevated in the different studies [20, 21, 22, 24, 39]. In the study by Jia et al. [21] the median serum level was as low as 24.5 (19.6-30.6) ng/mL in severe uncontrolled, treated asthmatic patients, while the average asthmatic serum level was 92.8 (± 38.4) ng/mL found by Kanemitsu et al. [20] and 76.5 (± 35.2) ng/mL found by Kim et al. [39], both examining more heterogeneous, smoking and co-morbid populations. In Kim's study [39] a cut-off value of 54.5 ng/mL discriminated asthmatic patients from healthy controls, with 71.8% sensitivity and 63.2% specificity (AUC = 0.75, $p < 0.001$). Our results are in line with these with a median value of 45.25 (32.67-64.55) ng/mL in the ANP group and 54.02 (44.30-74.94) in the AP group. Notably, in our study the disease was mainly well controlled and smoking was among the exclusion criteria in order to exclude the presence of COPD. In the ANP group the lung function of the patients was very good and asthma control was satisfactory.

We detected higher peripheral eosinophil percentage in both asthmatic groups, but there was no statistically significant difference (only a weak trend) between eosinophilic and non-eosinophilic pregnant patients regarding their periostin level. The previously reported correlation between periostin concentration and peripheral eosinophil percentage [20, 39] was detected as a trend in

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the pregnant asthmatic group in our study. In line with earlier results [40], peripheral eosinophil percentage correlated negatively with FEV_1 in both asthmatic groups.

Similarly to an earlier study [11] we measured peripheral CRP and IL-6 levels also, in order to have a more complete view on the inflammatory status of the patients. We observed slightly but clinically not relevantly elevated CRP levels in both asthmatic groups. Finally, IL-6 level was lower in the AP than in the ANP group, while in the earlier study IL-6 levels were comparable [11]. Lower IL-6 levels in AP women may be ascribed to pregnancy specific immune tolerance.

Limitations

The results should be interpreted with concern because there are several limitations of the study. The main limitation is the small sample size; however it is difficult to involve a large number of asthmatic pregnant women without comorbidities, concomitant medications, and without any known pregnancy complications. Our study had was designed to have a power of 80% to detect 50% difference among the 4 groups; however it must be noted, that because of the large dispersion in the periostin data published in previous articles (for example median values of 24.5 and 92.8 ng/mL), a priori power analysis may have carried some biases. Furthermore, the findings may differ in patients with severe asthma as the subjects in our study were mainly well controlled. Another limitation of the study is that lung function and asthma control data of some patients are missing (lung function in one AP, ACT in 2 ANP and 3 AP patients). Active smoking occurs in asthma including during pregnancy. As current smoking is reported to influence serum periostin concentrations [41], the circulating periostin concentrations may differ in asthmatic smokers during pregnancy. We compared our periostin data measured in plasma

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from EDTA samples to studies evaluating periostin in sera; however periostin levels have been earlier shown to be comparable within subjects within assay limits independently of matrix and anticoagulant (serum, plasma anticoagulated with EDTA, plasma anticoagulated with sodium citrate, and plasma anticoagulated with heparin) [21].

Conclusions

According to the data of this low sample-size study, pregnancy itself markedly increases circulating periostin levels, and this elevation is detectable both in healthy and asthmatic women. Hence, circulating periostin level in asthmatic and healthy pregnancy is similar. Although periostin correlates with lung function in asthmatic pregnancy, periostin as a biomarker has to be handled with caution in pregnant patients due to the influence of pregnancy on its plasma level. Further longitudinal studies with larger sample sizes would be warranted to show whether periostin is a suitable tool in phenotyping, control evaluation, or decision-making process during asthmatic pregnancy.

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References

1. Global Initiative for Asthma. Available: http://www.ginasthma.org. Accessed 10 February 2014.

2. Charlton RA, Hutchison A, Davis KJ, de Vries CS. Asthma management in pregnancy. PLoS One. 2013;8(4):e60247.

3. Demissie K, Breckenridge MB, Rhoads GG. Infant and maternal outcomes in the pregnancies of asthmatic women. Am J Respir Crit Care Med. 1998;158:1091–1095.

4. Breton MC, Beauchesne MF, Lemière C, Rey E, Forget A, Blais L. Risk of perinatal mortality associated with asthma during pregnancy. Thorax. 2009;64:101–106.

5. Murphy VE, Clifton VL, Gibson PG. Asthma exacerbations during pregnancy: incidence and association with adverse pregnancy outcomes. Thorax. 2006;61:169–176.

6. Tamási L, Horváth I, Bohács A, Müller V, Losonczy G, Schatz M. Asthma in pregnancy – Immunological changes and clinical management. Respir Med 2011;105:159–164.

7. Grindheim G, Toska K, Estensen ME, Rosseland LA. Changes in pulmonary function during pregnancy: a longitudinal cohort study. BJOG. 2012;119(1):94–101.

8. Tamási L, Bohács A, Bikov A, Andorka C, Rigó J Jr, Losonczy G, Horváth I. Exhaled nitric oxide in pregnant healthy and asthmatic women. J Asthma. 2009;46(8):786-791.

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9. 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. 2011;378(9795):983-990.

10. Nittner-Marszalska M, Liebhart J, Pawłowicz R, Kazimierczak A, Marszalska H, Kraus-Filarska M, et al. Fractioned exhaled nitric oxide (FE(NO)) is not a sufficiently reliable test for monitoring asthma in pregnancy. Nitric Oxide. 2013;33:56-63.

11. Ivancsó I, Toldi G, Bohács A, Eszes N, Müller V, Rigó J Jr, et al. Relationship of circulating soluble urokinase plasminogen activator receptor (suPAR) levels to disease control in asthma and asthmatic pregnancy. PLoS One. 2013;8(4):e60697.

12. Eszes N, Toldi G, Bohács A, Ivancsó I, Müller V, Rigó J Jr, et al. Relationship of circulating hyaluronic acid levels to disease control in asthma and asthmatic pregnancy. PLoS One 2014;9(4):e94678.

13. Parulekar AD, Atik MA, Hanania NA. Periostin, a novel biomarker of TH2-driven asthma. Curr Opin Pulm Med. 2014;20(1):60-5.

14. Liu AY, Zheng H, Ouyang G. Periostin, a multifunctional matricellular protein in inflammatory and tumor microenvironments. Matrix Biol. 2014;37C:150–156.

15. Takayama G, Arima K, Kanaji T, Toda S, Tanaka H, Shoji S, et al. Periostin: a novel component of subepithelial fibrosis of bronchial asthma downstream of IL-4 and IL-13 signals. J Allergy Clin Immunol. 2006;118(1):98–104.

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16. Masuoka M, Shiraishi H, Ohta S, Suzuki S, Arima K, Aoki S, et al. Periostin promotes chronic allergic inflammation in response to Th2 cytokines. J Clin Invest. 2012;122(7):2590–2600.

17. Blanchard C, Mingler MK, McBride M, Putnam PE, Collins MH, Chang G, et al. Periostin facilitates eosinophil tissue infiltration in allergic lung and esophageal responses. Mucosal Immunol. 2008;1(4):289–296.

18. Woodruff PG, Boushey HA, Dolganov GM, Barker CS, Yang YH, Donnelly S, et al. Genome-wide profiling identifies epithelial cell genes associated with asthma and with treatment response to corticosteroids. Proc Natl Acad Sci U S A. 2007;104(40):15858-63.

19. Sidhu SS, Yuan S, Innes AL, Kerr S, Woodruff PG, Hou L, et al. Roles of epithelial cell-derived periostin in TGF-beta activation, collagen production, and collagen gel elasticity in asthma. Proc Natl Acad Sci U S A. 2010;107(32):14170-5.

20. Kanemitsu Y, Matsumoto H, Izuhara K, Tohda Y, Kita H, Horiguchi T, et al. Increased periostin associates with greater airflow limitation in patients receiving inhaled corticosteroids. J Allergy Clin Immunol. 2013;132(2):305-12.e3.

21. Jia G, Erickson RW, Choy DF, Mosesova S, Wu LC, Solberg OD, et al. Periostin is a systemic biomarker of eosinophilic airway inflammation in asthmatic patients. J Allergy Clin Immunol. 2012;130(3):647–654.e10.

22. Corren J, Lemanske RF, Hanania NA, Korenblat PE, Parsey MV, Arron JR, et al. Lebrikizumab treatment in adults with asthma. N Engl J Med. 2011;365(12):1088–1098.

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23. Arron JR, Choy DF, Scheerens H, Matthews JG. Noninvasive biomarkers that predict treatment benefit from biologic therapies in asthma. Ann Am Thorac Soc. 2013;10 Suppl:S206-13.

24. Song JS, You JS, Jeong SI, Yang S, Hwang IT, Im YG, et al. Serum periostin levels correlate with airway hyperresponsiveness to methacholine and mannitol in children with asthma. Allergy. 2015;doi:10.1111/all.12599.

25. Nagasaki T, Matsumoto H, Kanemitsu Y, Izuhara K, Tohda Y, Horiguchi T, et al. Using exhaled nitric oxide and serum periostin as a composite marker to identify severe/steroid-insensitive asthma. Am J Respir Crit Care Med. 2014;190(12):1449-52.

26. Réger B, Péterfalvi A, Litter I, Pótó L, Mózes R, Tóth O, et al. Challenges in the evaluation of D-dimer and fibrinogen levels in pregnant women. Thromb Res. 2013;131(4):e183-7.

27. van den Broe NR, Letsky EA. Pregnancy and the erythrocyte sedimentation rate. BJOG. 2001;108(11):1164-7.

28. Valdimarsson H, Mulholland C, Fridriksdottir V, Coleman DV. A longitudinal study of leucocyte blood counts and lymphocyte responses in pregnancy: a marked early increase of monocyte-lymphocyte ratio. Clin Exp Immunol. 1983;53(2):437-43.

29. Watts DH, Krohn MA, Wener MH, Eschenbach DA. C-reactive protein in normal pregnancy. Obstet Gynecol. 1991;77(2):176-80.

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30. Hiroi H, Momoeda M, Nakazawa F, Koizumi M, Tsutsumi R, Hosokawa Y, et al. Expression and regulation of periostin/OSF-2 gene in rat uterus and human endometrium. Endocr J. 2008;55(1):183–189.

31. Ahn HW, Farmer JL, Bazer FW, Spencer TE. Progesterone and interferon tau-regulated genes in the ovine uterine endometrium: identification of periostin as a potential mediator of conceptus elongation. Reproduction. 2009;138(5):813–825.

32. Rios H, Koushik SV, Wang H, Wang J, Zhou HM, Lindsley A, et al. periostin null mice exhibit dwarfism, incisor enamel defects, and an early-onset periodontal disease-like phenotype. Mol Cell Biol. 2005;25(24):11131–44.

33. Zhu S, Barbe MF, Amin N, Rani S, Popoff SN, Safadi FF, et al. Immunolocalization of Periostin-like factor and Periostin during embryogenesis. J Histochem Cytochem. 2008;56(4):329–345.

34. Snider P, Hinton RB, Moreno-Rodriguez RA, Wang J, Rogers R, Lindsley A, et al. Periostin is required for maturation and extracellular matrix stabilization of noncardiomyocyte lineages of the heart. Circ Res. 2008;102(7):752–760.

35. Ahlfeld SK, Gao Y, Wang J, Horgusluoglu E, Bolanis E, Clapp DW, et al. Periostin downregulation is an early marker of inhibited neonatal murine lung alveolar septation. Birth Defects Res A Clin Mol Teratol. 2013;97(6):373–385.

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36. Morelli M, Misaggi R, Di Cello A, Zuccalà V, Costanzo F, Zullo F, et al. Tissue expression and serum levels of periostin during pregnancy: a new biomarker of embryo-endometrial cross talk at implantation. Eur J Obstet Gynecol Reprod Biol. 2014;175:140–144.

37. Sasaki H, Roberts J, Lykins D, Fujii Y, Auclair D, Chen LB. Novel chemiluminescence assay for serum periostin levels in women with preeclampsia and in normotensive pregnant women. Am J Obstet Gynecol. 2002;186(1):103–108.

38. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J. 2005;26:319–338.

39. Kim MA, Izuhara K, Ohta S, Ono J, Yoon MK, Ban GY, et al. Association of serum periostin with aspirin-exacerbated respiratory disease. Ann Allergy Asthma Immunol. 2014;113(3):314–320.

40. Tajiri T, Matsumoto H, Niimi A, Ito I, Oguma T, Nakaji H, et al. Association of eosinophilic inflammation with FKBP51 expression in sputum cells in asthma. PLoS One. 2013;8(6):e65284.

41. Thomson NC, Chaudhuri R, Spears M, Haughney J, McSharry C. Serum periostin in smokers and never smokers with asthma. Resp Med 2015;109(6):708-715.

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Table 1. Clinical data and inflammatory parameters of the four study groups (mean±SD, median [interquartile range]).

	HNP(n = 12)	**HP(n = 17)**	**ANP(n = 19)**	**AP(n = 14)**
Age (years)	31.4±6.9	34.8±3.2[n = 15]	35.0±6.3	31.1±6.2
Gestational age at sampling (weeks)	-	29.0±10.9	-	27.3±8.3
1. / 2. / 3. trimester	-	3 / 2 / 12	-	1 / 4 / 9
Gestational age at delivery (weeks)	-	38.5±1.9[n = 14]	-	37.9±1.4[n = 8]
Fetal birth weight (grams)	-	3309±458.0[n = 14]	-	3023±502.8[n = 8]
FEV_1 (% of predicted)	-	-	98.3±15.1[n = 18]	88.5±13.9
FVC (% of	-	-	108.3±11.6[n = 18]	95.5±10.3[c]

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predicted)				
PEF (% of predicted)	-	-	90.9±13.9[n = 18]	77.9±15.8[n = 13, c]
R_{aw}	-	-	0.29±0.15[n = 18]	0.32±0.19
$FEF_{25-75\%}$ (% of predicted)	-	-	80.5±25.2[n = 18]	76.50±27.9[n = 12]
ACT total score	-	-	21.65±3.39[n = 17]	20.18±4.12[n = 11]
Daily dose of ICS (beclomethasone equivalent, µg)	-	-	800 (0- 1000)[n = 18]	650 (0- 1000)
Steroid-naïve / steroid-treated patients	-	-	6 / 13	6 / 8
Eosinophil cells (%)	1.55 [1.26-2.02]	0.88 [0.50-1.00][n = 7]	2.51 [2.09-4.30][n = 18, b]	2.26 [1.37-4.16][b]
CRP (mg/L)	3.0 [1.3-3.0] [n = 11]	-	4.0 [3.0-5.5][n = 17, a]	6.0 [3.0-6.5][n = 13, a]

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IL-6 (pg/mL)	-	-	4.21 [1.89-6.95]$^{n = 17}$	1.93 [1.50-3.34]$^{n = 13, c}$
Periostin (ng/mL)	55.68 [37.21-67.20]	68.81 [57.34-98.84]^{a}	45.25 [32.67-64.55]^{b}	54.02 [44.30-74.94]^{c}

HNP -- healthy non-pregnant; HP -- healthy pregnant; ANP -- asthmatic non-pregnant; AP -- asthmatic pregnant; FEV_1 -- forced expiratory volume in 1 second; FVC -- forced vital capacity; PEF -- peak expiratory flow rate; R_{aw} -- airway resistance; $FEF_{25-75\%}$ -- forced expiratory flow at 25--75% of forced vital capacity; ACT -- asthma control test; ICS -- inhaled corticosteroid; CRP -- C-reactive protein; IL-6 -- interleukin 6; ^{a} $p < 0.05$ vs. HNP, ^{b} $p < 0.05$ vs. HP, ^{c} $p < 0.05$ vs. ANP.

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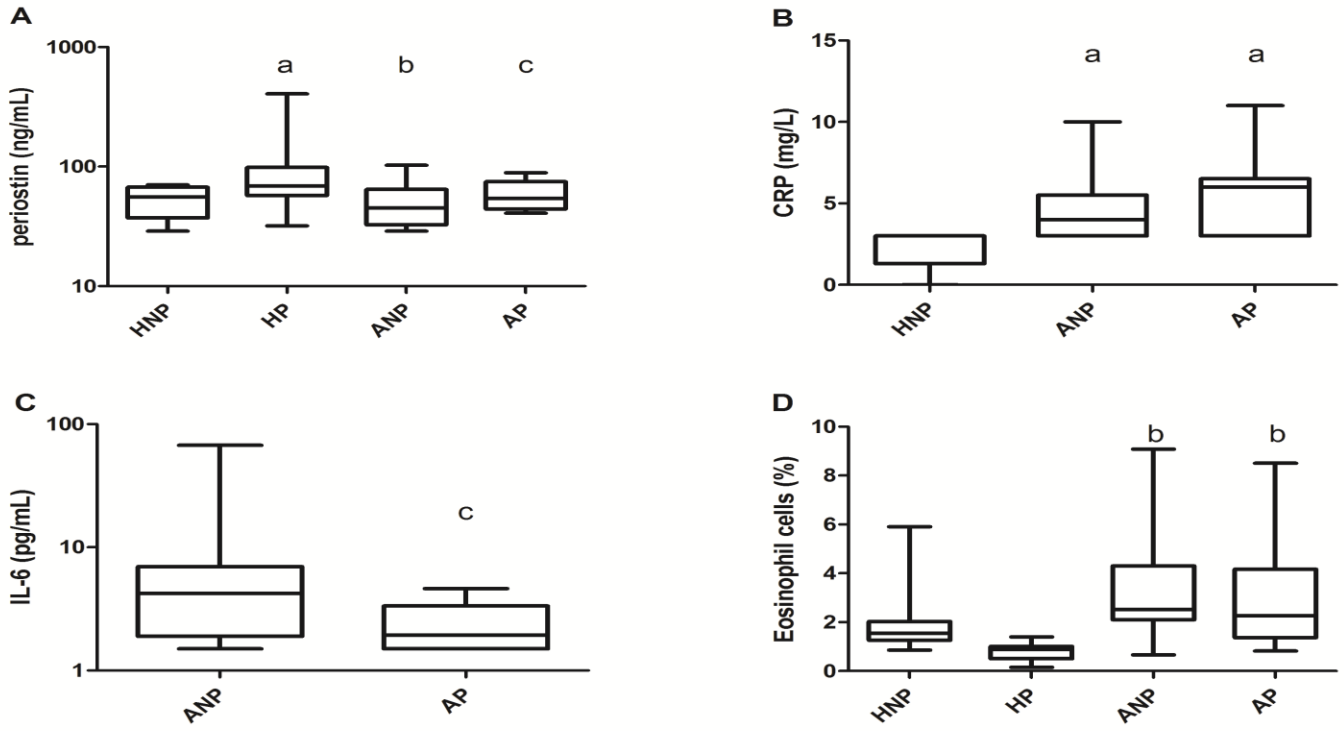

Figure 1. Circulating periostin (A), CRP (B), IL-6 (C) levels and peripheral eosinophil cell percentage (D) measured in healthy non-pregnant and pregnant, and asthmatic non-pregnant and pregnant subjects. HNP -- healthy non-pregnant; HP -- healthy pregnant; ANP -- asthmatic non-pregnant; AP -- asthmatic pregnant; CRP -- C-reactive protein; IL-6 -- interleukin 6; [a] $p < 0.05$ vs. HNP, [b] $p < 0.05$ vs. HP, [c] $p < 0.05$ vs. ANP.

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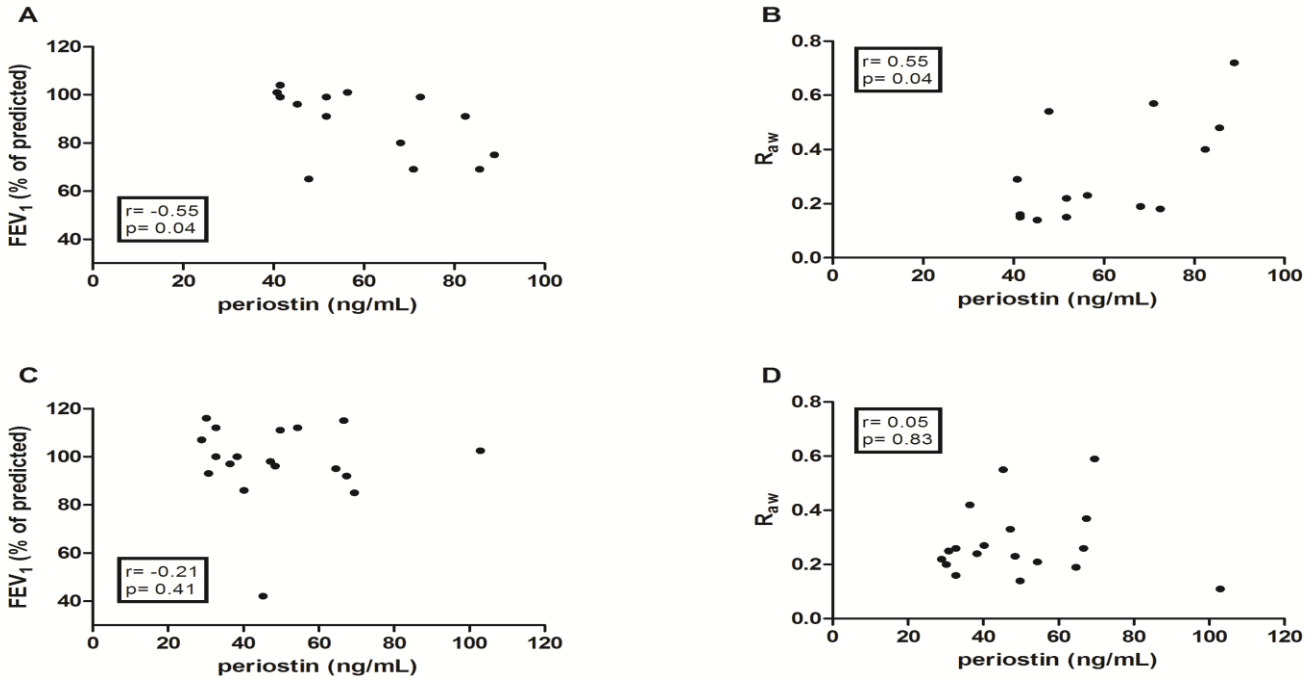

Figure 2. Negative correlation of circulating periostin to FEV_1 (A) and positive correlation of circulating periostin to airway resistance (R_{aw}) (B) in asthmatic pregnant patients, and lack of these correlations (C, D) in asthmatic non-pregnant patients FEV_1 -- forced expiratory volume in 1 second; R_{aw} -- airway resistance.

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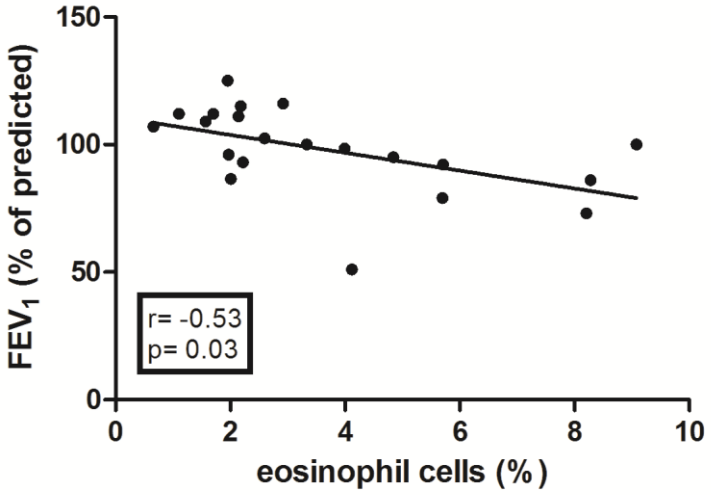

Figure 3. Negative correlation of peripheral eosinophil cell percentage to FEV_1 in asthmatic non-pregnant patients. FEV_1 -- forced expiratory volume in 1 second.

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