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RESEARCH ARTICLE

Circulating and exhaled vascular endothelial growth factor in asthmatic pregnancy

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Abstract

Context: Vascular endothelial growth factor (VEGF) plays a role in asthma and pathological pregnancies.

Objective: This is the first study assessing plasma and exhaled breath condensate VEGF levels in asthmatic pregnancy.

Material and methods: Thirty-one asthmatic pregnant, 29 asthmatic nonpregnant, 28 healthy pregnant and 22 healthy nonpregnant women were enrolled. Plasma was collected in all subjects, EBC in 57 volunteers for VEGF measurements.

Results: Plasma VEGF decreased in both pregnant groups ($p < 0.01$), without any differences between the asthmatic and the respective nonasthmatic groups ($p > 0.05$). VEGF was undetectable in EBC.

Conclusion: Concomitant asthma does not affect plasma VEGF during pregnancy.

Keywords: Asthma, breath test, pregnancy, vascular endothelial growth factor

Introduction

Asthma is one of the most common medical conditions complicating pregnancy, which represents a risk factor for several maternal and fetal complications (Dombrowski 2006; NAEPP expert panel report, 2005; Tamási et al. 2011). Furthermore, pregnancy may also deteriorate the previously stable asthma by increasing the frequency of asthma exacerbations and hospitalizations due to asthma attacks (Schatz 1999). While the course of asthma during pregnancy is unpredictable, an optimal asthma control was associated with decreased maternal and fetal risks for complications (Belanger et al. 2010). We have found signs of fetal growth restriction being related to active, asthma-associated maternal immune reactions (Tamási et al. 2005; Bohács et al. 2010) previously. These results suggest a promising role for the assessment of circulating biomarkers to predict pregnancy outcomes. One potential candidate could be the vascular endothelial growth factor (VEGF) as it was associated with the pathophysiology of asthma and pathological pregnancies as well, by several lines of evidence.

VEGF plays a critical role in the normal embryonic and placental angiogenesis (Espinoza et al. 2010). However, the production of a soluble VEGF receptor, the fms-like tyrosine kinase 1 (sFlt1) increases during pregnancy (Clark et al. 1998; Banks et al. 1998). This molecule inhibits the function of VEGF (Maynard et al. 2003) and it may interfere with commercially available ELISA kits by blocking the VEGF-antibody binding site (Jelkmann 2001). In this case the measured levels reflect "free" rather than "total" VEGF. VEGF and sFlt1 levels were shown to be altered in obstetrical complications (Levine et al. 2004; Park et al. 2005; Espinoza et al. 2010; Molvarec et al. 2010), which suggests that the physiological levels of VEGF are tightly regulated in pregnancy.

Asthma was accompanied by elevated VEGF levels in airway samples, including induced sputum (Asai et al. 2002; Kanazawa et al. 2002b; Asai et al. 2003),

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bronchoalveolar lavage (BAL; Feltis et al. 2006) and bronchial biopsies (Hoshino et al. 2001a, 2001b). Furthermore, a significant correlation was found between airway VEGF levels and the severity of bronchial obstruction in asthmatic patients (Hoshino et al. 2001b; Asai et al. 2003), while treatment with inhaled corticosteroids (ICS; Asai et al. 2002; Asai et al. 2003) reduced its airway concentrations.

However, the airway sampling techniques applied in those studies are either semi-invasive or invasive; therefore they cannot be utilized in pregnant subjects. Exhaled breath condensate (EBC) is a completely harmless, noninvasive method to study the airways (Horváth et al. 2005). During sampling, airway molecules trap in the moisture of exhaled breath which condensates in a cooled chamber. While this technique is promising, some factors may limit its widespread use. For example, the concentrations of EBC biomolecules are near to the detection limit of analytical techniques. Still, numerous mediators were identified in EBC successfully, including VEGF (Leung et al. 2005; Dalaveris et al. 2009; Gessner et al. 2010; Kastelijn et al. 2010).

The aim of this study was to analyze whether asthma alters circulating and EBC VEGF levels in pregnant women. In addition, we analyzed if the alterations in plasma VEGF are due to the modified VEGF-binding capacity.

Methods

Study subjects

Thirty-one asthmatic pregnant (AP; 31 ± 5 years), 29 asthmatic nonpregnant (ANP; 32 ± 8 years), 28 healthy pregnant (HP; 31 ± 5 years) and 22 healthy nonpregnant (HNP; 31 ± 5 years) women participated in the study. Asthmatic patients were enrolled at the outpatient clinic of the Department of Pulmonology. The AP group consisted of 24 pregnant women in their second trimester (20 ± 5 gestational weeks) and seven subjects in their third trimester (36 ± 3 gestational weeks). Furthermore, nine second-trimester AP subjects were reinvited for blood sampling in their third trimester to assess temporal changes along trimesters. Sixteen AP patients used ICS, while fifteen were steroid-naive and used short-acting β2-agonists in case of breathlessness. Twenty-two ANP patients were on prescribed ICS therapy and seven were steroid-naive. The asthma in AP and ANP groups was diagnosed according to the Global Initiative for Asthma guidelines (Global Initiative for Asthma, 2009) and all patients were partially or well controlled. HP subjects were recruited when attending their scheduled visit at the First Department of Gynecology and Obstetrics. All HP subjects were in their second trimester (23 ± 4 gestational weeks). Asthma was excluded after examination by a respiratory medicine specialist in the HP and HNP groups.

None of the subjects had diabetes mellitus, liver or renal failure or had respiratory tract infection within the 4 weeks preceding the study. Current smokers or ex-smokers with a history of >5 pack years were excluded. None of the AP and HP subjects had any obstetrical disorders complicating pregnancy. In addition, patients with having acute exacerbation due to asthma in the year prior to the study were also excluded. Patients' characteristics are summarized in Table 1.

The study was approved by the Semmelweis University Ethics Committee (TUKEB 110/2007), and all patients gave written informed consent prior to participation in the study.

Study design

The study had a cross-sectional, case–control design. From all subjects venous blood and from 57 subjects (19 AP, 9 ANP, 18 HP and 11 HNP) EBC was collected for VEGF measurements. To investigate the VEGF/sFlt1 balance in blood, we additionally measured sFlt1 in 85 plasma samples (21 AP, 23 ANP, 21 HP and 20 HNP). This mediator was analyzed only in the second-trimester subjects (Palm et al. 2011).

Additionally, lung function and fractional nitric oxide measurements were performed in asthmatic subjects. All patients were instructed not to use their medications for at least 12 h before sampling.

The sample size was estimated for the main aim to identify differences between groups in plasma VEGF with an effect size of 0.40 and $\beta = 0.90$ power assuming the nonparametric data distribution.

Sample collection and measurement

Venous blood was collected in EDTA tubes and processed as reported in the ELISA kit guideline. The samples were stored at −80°C until the analysis using commercially available assays (DVE00 and DVR100B, VEGF and sFlt1, respectively; R&D Systems, Abingdon, UK). The detection limits for plasma VEGF and sFlt1 were 9 and 2.5 pg/mL, respectively. To study the interference between sFlt1 and the VEGF ELISA kit, we plotted VEGF standard curve (ranging from 0 to 500 pg/mL) in a presence of different amounts of sFlt1 (4000, 2000, 1000, 500 and 250 pg/mL).

EBC samples were collected using Rtube (Respiratory Research, Charlottesville, VA, USA). The chilling tube was held at −80°C and condensate was collected for 10 min without wearing nose clip. The samples were stored at −80°C until measurement with the same VEGF ELISA kit as used for plasma samples without pretreatment. The detection limit for EBC VEGF was 5 pg/mL (estimated by the +2SD variation of five parallel 0 pg/mL concentration points). In addition, to ensure that proteins are present in EBC samples, total protein was measured in 14 AP, 6 ANP, 13 HP and 7 HNP subjects using Roti-Quant Universal colorimetric assay kit (Roth GMBH, Karlsruhe, Germany).

Lung function and exhaled nitric oxide measurements

Lung function tests were performed using an electronic spirometer (PDD-301/s, Piston, Budapest, Hungary), according to the latest guideline (Miller et al. 2005). Fractional exhaled

Table 1. Subject characteristics.

Characteristics	AP ($N = 31$)	ANP ($N = 29$)	HP ($N = 28$)	HNP ($N = 22$)
Age, years	31 ± 5	32 ± 8	31 ± 5	31 ± 5
Gestational weeks at the time of sampling	23 ± 8	NA	23 ± 4	NA
Neonatal birth weight, g	3493 ± 664	NA	3373 ± 326	NA
FEV_1, L (% predicted)	2.8 ± 0.4 (88 ± 11)	2.9 ± 0.6 (89 ± 17)	ND	ND
FVC, L (% predicted)	3.6 ± 0.5 (97 ± 12)	3.7 ± 0.7 (101 ± 15)	ND	ND
FEV_1/FVC	0.79 ± 0.08	0.77 ± 0.09	ND	ND
$FE_{NO,\ ppb}$	20 (9–115)	16 (5–82)	ND	ND
ICS dose, μg BDP equivalent	310 ± 445	410 ± 295	NA	NA

ANP, asthmatic nonpregnant; AP, asthmatic pregnant; BDP, beclomethasone dipropionate; FE_{NO}, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; HNP, healthy nonpregnant; HP, healthy pregnant; ICS, inhaled corticosteroid; NA, not applicable; ND, not determined.

nitric oxide (FE_{NO}) was measured by a NIOX MINO electrochemical analyzer (Aerocrine; Solna, Sweden) at an expiratory flow of 50 mL/s as per guideline recommendations.

Statistical analysis

We used Graphpad Prism 4.0 (GraphPad Software Inc., San Diego, CA, USA) for statistical analysis. The normality distribution of the data was assessed by Kolmogorov–Smirnov test. Plasma and EBC VEGF as well as plasma sFlt1 concentrations were compared among groups using Kruskal–Wallis test and Dunns *post hoc* tests. The effect of gestational age on plasma VEGF was analyzed using Mann–Whitney, Wilcoxon and Spearman tests. The relationships between VEGF levels and clinical parameters in the asthmatic groups were assessed by Spearman's tests. Since plasma and condensate VEGF, plasma sFlt1, as well as FE_{NO} levels were not normally distributed, these variables were expressed as median (range), otherwise as mean ± SD. $p < 0.05$ was considered significant.

Results

Clinical data

There was no difference in forced expiratory volume in one second (FEV_1; 2.8 ± 0.4 L vs. 2.9 ± 0.6L; AP vs. ANP, respectively) or forced vital capacity (FVC; 3.6 ± 0.5 L vs. 3.7 ± 0.7 L; AP vs. ANP, respectively) values or FE_{NO} levels (20 [9–115] ppb vs. 16 [5–82] ppb; AP vs. ANP, respectively) between the two asthmatic groups ($p > 0.05$). The ICS dose was similar in the two groups (310 ± 445 μg vs. 410 ± 295 μg, beclomethasone dipropionate equivalent, $p = 0.16$; AP vs. ANP, respectively).

In both pregnant groups the pregnancy and the labor were uncomplicated, the birth weight (3493 ± 664 g and 3373 ± 326 g, $p = 0.37$; AP and HP, respectively) and the gestational week at delivery (39 ± 2 and 39 ± 1 weeks, $p = 0.80$; AP and HP, respectively) were similar. There was no difference in the 0-min (8.9 ± 0.8 and 9.1 ± 0.5, $p = 0.34$; AP and HP, respectively) or the 5-min (9.9 ± 0.3 and 9.9 ± 0.2, $p = 0.64$; AP and HP, respectively) Apgar scores between the two groups.

Interference of sFlt1 with VEGF assay

Compared to the sFlt1 free VEGF standard curve, the presence of sFlt1, even at 250 pg/mL, reduced VEGF levels in the assay we used. In addition, 62.5 pg/mL was the minimally detectable VEGF concentration at 250 pg/mL sFlt1. Because the ELISA kit detects only the unbound VEGF, our results reflect free VEGF.

Free plasma VEGF results

Free VEGF was detectable in 78 samples (71%); 18 (58%) in AP, 29 (100%) in ANP, 10 (36%) in HP and 20 (91%) in HNP samples with significant differences among groups (14 [0–161], 81 [21–826], 0 [0–184] and 77 [0–458] pg/mL; AP, ANP, HP and HNP, respectively, $p < 0.001$, Figure 1). Significant differences were observed between AP and ANP ($p < 0.001$) and HP and HNP groups ($p < 0.001$) with decreased levels of free VEGF in the two pregnant groups. However, free VEGF levels were similar when the AP and HP or the ANP and HNP groups were compared ($p > 0.05$). There was no difference between steroid-naive AP versus HP groups ($p = 0.51$) and steroid-naive ANP vs. HNP ($p = 0.80$) groups. In addition, no difference was observed between AP and HP groups when only the second-trimester women were analyzed ($p = 0.11$).

Free VEGF levels were similar between second- and third-trimester AP subjects (18 [0–161] vs. 0 [0–95] pg/mL; second vs. third trimester, respectively, $p = 0.54$, Figure 2A). There was no temporal change between second- and third-trimester VEGF levels in samples of the nine AP women (45 [0–161] and 20 [0–909] pg/mL, second and third trimester, respectively, $p = 0.67$, Figure 2B). We found no significant association between free plasma VEGF levels and gestational weeks at the time of sampling in the AP ($p = 0.70$) or in the HP group ($p = 0.89$).

In the AP group, there was a significant inverse relationship between free plasma VEGF and FE_{NO} levels ($p = 0.01$, $r = -0.51$), a marker of airway inflammation while no other clinical variable was related to free VEGF levels. The relationship between free VEGF and FE_{NO} was absent after adjustment on ICS dose ($p = 0.22$), suggesting steroid-dependence of FE_{NO}. Free VEGF levels in steroid treated and steroid-naive AP patients were similar (33 [0–161] vs. 7 [0–141] pg/mL, steroid-treated vs. steroid-naive, $p = 0.19$), and no significant relationship was observed between free plasma VEGF and ICS dose in AP patients ($p = 0.27$).

In the ANP group, we found no relationship between free plasma VEGF and any of the clinical parameters ($p > 0.05$) and no difference in free VEGF concentration between steroid treated and steroid-naive patients ($p = 0.87$).

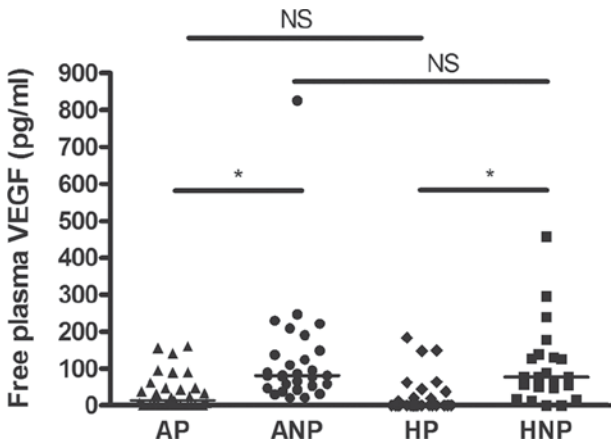

Figure 1. Comparison of free plasma vascular endothelial growth factor levels among groups. Significant differences were observed in free plasma vascular endothelial growth factor (VEGF) levels among the four groups ($p < 0.05$). Free plasma VEGF levels and median values of groups are expressed. AP, asthmatic pregnant, triangles; ANP, asthmatic nonpregnant, circles; HP, healthy pregnant, diamonds; HNP, healthy nonpregnant, squares. *$p < 0.05$.

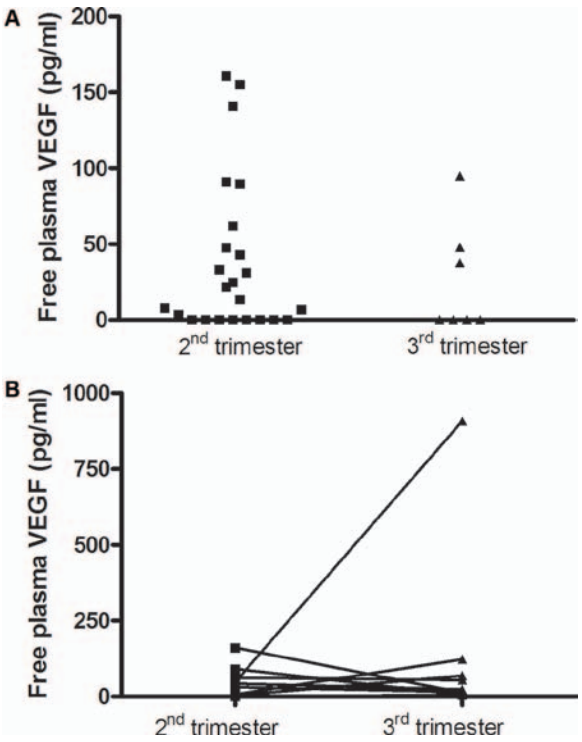

Figure 2. Comparison of free plasma vascular endothelial growth factor levels between second- and third-trimester asthmatic pregnant subjects. No difference was observed in free plasma vascular endothelial growth factor levels between the second (squares) and third (triangles) trimesters neither when the second- and third-trimester asthmatic pregnant were compared (A) nor when the temporal changes were assessed in nine asthmatic pregnant women (B).

EBC VEGF and total protein results

VEGF was measurable above the detection limit only in 13 samples (5 AP, 3 ANP, 4 HP and 1 HNP) which means that VEGF was detectable in overall of 23% of the samples. The VEGF concentrations in condensate samples were 0 (0–93), 0 (0–74) , 0 (0–52) and 0 (0–7) pg/mL (AP, ANP, HP and HNP, respectively, Figure 3). There was no difference among groups in EBC VEGF levels ($p = 0.74$).

Proteins were detectable in 12 AP (86%), 4 ANP (67%), 10 HP (77%) and 7 HNP (100%) samples (the detection limit was 1 μg/mL). There was no difference in total protein levels between groups (8 [0–218], 17 [0–33], 5 [0–76] and 8 [2–18] μg/mL; AP, ANP, HP and HNP, respectively, $p = 0.67$).

Plasma sFlt1 results

There was a significant difference in plasma sFlt1 levels among the four groups ($p < 0.001$, Figure 4), with plasma sFlt1 concentrations of 1544 [840–3484], 44 [19–83], 1508 [764–4192] and 36 [10–98] pg/mL [AP, ANP, HP and HNP, respectively]. sFlt1 levels were elevated in AP and HP compared to ANP and HNP groups ($p < 0.001$) without any differences between AP vs. HP or ANP vs. HNP groups ($p > 0.05$). No correlation was found between sFlt1 levels and any of the clinical parameters in AP or ANP groups.

Relationship of plasma VEGF or sFlt1 with neonatal birth weight

There was no correlation between free VEGF and birth weight ($p = 0.70$ and $p = 0.28$; AP and HP, respectively) or sFlt1 and birth weight ($p = 0.71$ and $p = 0.47$; AP and HP, respectively) in either group.

Discussion

In this study, plasma and EBC VEGF were analyzed in asthmatic pregnancy. We found that asthma does not affect free plasma VEGF levels in nonpregnant women; however free VEGF is not measurable in pregnancy possibly due to the increased concentrations of sFlt1. We have also demonstrated that VEGF is hardly detectable in EBC.

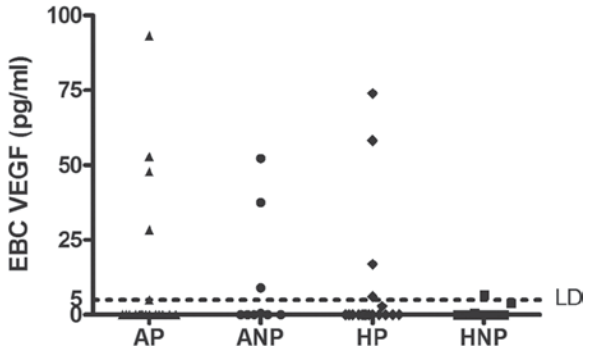

Figure 3. Comparison of exhaled breath condensate vascular endothelial growth factor levels among groups. No difference was observed in exhaled breath condensate vascular endothelial growth factor levels among the four groups. The majority of the values were below the lower limit of detection (LD, 5 pg/mL).

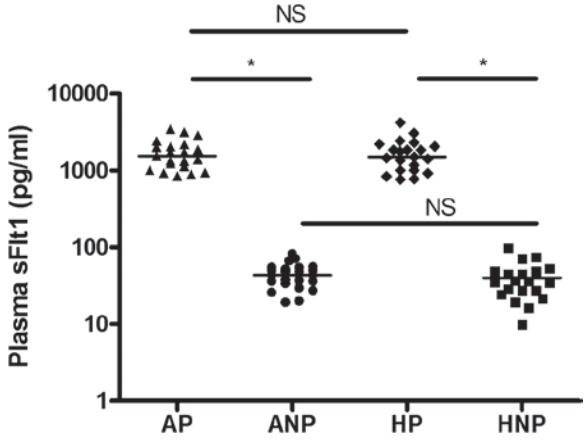

Figure 4. Comparison of plasma soluble fms-like tyrosine kinase 1 levels among the four groups. Significant differences were observed in plasma soluble fms-like tyrosine kinase 1 among the four groups ($p < 0.05$). Data are expressed in a logarithmic range with medians. AP, asthmatic pregnant, triangles; ANP, asthmatic nonpregnant, circles; HP, healthy pregnant, diamonds; HNP, healthy nonpregnant, squares. *$p < 0.05$.

Asthma is one of the most common disorders which may complicate pregnancy. VEGF had a potential to be a biomarker for this condition as it was suggested to play a role in asthma pathogenesis. VEGF was also shown to be altered in various obstetrical complications, such as pre-eclampsia and may have a diagnostic value in these conditions (Levine et al. 2004; Kharb 2009). However, during physiological pregnancy the biologically active free VEGF levels are low and hardly measurable in plasma. Hence, the analysis of circulating sFlt1 is suggested in parallel with VEGF, as its concentration is well above detection limit during pregnancy. The sFlt1 is the soluble form of Flt1 or VEGF receptor1, albeit without any tyrosine kinase activity. Therefore after binding circulating VEGF, the molecule does not induce any effect, thus sFlt regulates the physiological role of VEGF by inhibition (Maynard et al. 2003). It was shown that while VEGF does not change, there is a significant temporal course of sFlt1 during pregnancy. It increases initially after conception and remains constant until around 30th gestational weeks. Then it starts to increase rapidly with a peak at terminus (Levine et al. 2004; Palm et al. 2011). However, an increase in circulating sFlt1 earlier than the 30th week in parallel with a reduction in free VEGF was associated with pre-eclampsia. The impaired angiogenic VEGF and angiostatic sFlt1 balance observed in pre-eclampsia is not surprising, as VEGF has an essential role in fetal and placental angiogenesis, and it is well known, that pre-eclampsia is associated with abnormal placental vasculature.

Similarly to pre-eclampsia, an altered vasculature is also present in bronchial vessels of asthma. It has been known that the hyperemia of the bronchial wall contributes to the pathogenesis of this disease by several lines of evidence. The increased vessel size, number of bronchial vessels and vascular surface area as well as the resulting edema might physically compress the airways (Charan et al. 1997). However the association between increased bronchial blood flow and airway narrowing is more likely due to the bronchial smooth muscle hyperresponsiveness caused by plasma leakage (Van de Graaf et al. 1991; Kanazawa et al. 2002a). The role of VEGF in the development of vascular changes in asthmatics is supported by several studies (Hoshino et al. 2001a, 2001b; Lee et al. 2004). In addition, VEGF causes eosinophil chemotaxis (Asai et al. 2003), and asthma-related cytokines (IL-4, IL-5 and IL-13) and cysteinyl leukotrienes induce the production of VEGF in airway smooth muscle cells (Corne et al. 2000; Poulin et al. 2011).

Increased levels of VEGF were measured in various airway samples, including induced sputum (Asai et al. 2002; Kanazawa et al. 2002b; Asai et al. 2003) and BAL (Feltis et al. 2006). However, to our knowledge, only one study has investigated the levels of circulating VEGF in asthmatics, so far (Lee et al. 2008), finding significantly increased plasma VEGF levels in stable asthmatics; however the number of subjects in that study was threefold lower compared to ours. On contrary to the recent investigation, we did not find any significant alterations between asthmatic and nonasthmatic groups compared either in pregnant or nonpregnant state. One possible explanation for the controversy might be the small number of patients. Of note, in our study we also found few extremely high plasma VEGF values, which were not explainable by the current clinical status. This suggests that there might be some unidentified factors which affecting the results lead to contradictions between studies.

However, it is implausible that the negative results between asthmatic and control groups in plasma VEGF in our study are due to the steroid treatment caused attenuation, as we have shown that no difference existed in plasma VEGF levels between steroid-treated and steroid-naive patients and no relationship was observed between plasma VEGF concentrations and ICS dose. Nevertheless, our results suggest that stable, partially or well controlled asthma does not compromise the clinical utility of free VEGF measurements during pregnancy. On the other hand, studies aiming to investigate the role of VEGF in the pathophysiology of asthma should be carried out in airway samples, as systemic specimens do not fully represent airway processes. However, BAL or induced sputum are invasive or semi-invasive techniques which carry potential risks for side effects, therefore they cannot be applied in pregnant subjects.

Exhaled breath analysis is a promising and harmless technique for sampling the airway tract and to assess inflammatory processes during gestation (Horváth et al. 2005; Tamási et al. 2009; Bikov et al. 2011). To this date few studies have detected VEGF in EBC (Leung et al. 2005; Dalaveris et al. 2009; Kastelijn et al. 2010; Gessner et al. 2010; Rodríguez-Trigo et al. 2010; Font-Ribera et al. 2010), and only one of them was performed in asthmatic patients (Leung et al. 2005). The results of the latter study were similar to ours, as the overall detection rate was small (41% [Leung et al. 2005] vs. 23% observed by us) with no

differences between asthmatic and nonasthmatic groups. Furthermore, VEGF levels were found in very low concentrations (5–10 pg/mL) in healthy, chronic obstructive pulmonary disease and lung-transplanted subjects and were well above the detection limit only in lung cancer patients (Gessner et al. 2010). However, detection limits may vary between measuring techniques, and multiplex bead arrays (detection limit: 0.1–2.5 pg/mL) were suggested to be more sensitive tools to detect VEGF than commercially available ELISAs. Still, in agreement with the previous studies, we showed that the EBC may not be an applicable sample collection method for VEGF detection. To ensure that proteins are captured in the EBC fluid, we measured total protein levels in our EBC samples. In overall, proteins were detectable in 83% of the samples, which suggests that the lack of VEGF measurability was not due to the inability to capture proteins in the condensate fluid. Of note, we cannot rule out the possibility that the failure in detection of VEGF in EBC was due to the increased levels of sFlt1 in EBC samples. Unfortunately, we did not have enough condensate volume to perform sFlt1 measurements in parallel with VEGF.

In our study we found decreased levels of free plasma VEGF in the two pregnant groups, with less than half of the samples detectable during gestation, which is probably due to the increased sFlt1 concentrations. However, in addition to reducing the levels of free VEGF, in the presence of 250 pg/mL sFlt1 the lower detection limit of VEGF ELISA raised to 62.5 pg/mL, therefore plasma VEGF measurements may be not informative during gestation. There was no association between free VEGF levels and gestation age in the AP group, which is in agreement with previous studies in non-AP women (Levine et al. 2004; Palm et al. 2011). On the other hand, we succeeded to determine sFlt1 concentration in all the plasma samples. Because of the relevant interference of sFlt1 with VEGF ELISA kit, we suggest to measure sFlt1 instead of VEGF for the assessment of angiogenic/angiostatic balance during gestation. To our knowledge, this is the first study to measure sFlt1 in asthma. Our results indicate that asthma does not change the levels of circulating sFlt1 neither in pregnant or nonpregnant subjects. These results are important when conducting large studies assessing the clinical utility of sFlt1 measurements to determine obstetrical complications. Unfortunately, we did not have enough EBC volume to measure sFlt1 in condensate samples, therefore further studies are needed to evaluate the usefulness of this mediator in investigation of airway processes.

Conclusion

In summary, we have shown that partially or well controlled, stable asthma does not affect the circulating free VEGF and sFlt1 levels in pregnancy. We cannot exclude that local VEGF alterations might happen in the airways of AP subjects, but the currently available airway sampling techniques (e.g. induced sputum, BAL and bronchoscopy) are not suitable in pregnant subjects due to their side effects. Developing more sensitive techniques to detect low VEGF levels in EBC would be promising.

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Declaration of interest

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References

Asai K, Kanazawa H, Kamoi H, Shiraishi S, Hirata K, Yoshikawa J. (2003). Increased levels of vascular endothelial growth factor in induced sputum in asthmatic patients. *Clin Exp Allergy* 33:595–599.

Asai K, Kanazawa H, Otani K, Shiraishi S, Hirata K, Yoshikawa J. (2002). Imbalance between vascular endothelial growth factor and endostatin levels in induced sputum from asthmatic subjects. *J Allergy Clin Immunol* 110:571–575.

Banks RE, Forbes MA, Searles J, Pappin D, Canas B, Rahman D, Kaufmann S, Walters CE, Jackson A, Eves P, Linton G, Keen J, Walker JJ, Selby PJ. (1998). Evidence for the existence of a novel pregnancy-associated soluble variant of the vascular endothelial growth factor receptor, Flt-1. *Mol Hum Reprod* 4:377–386.

Belanger K, Hellenbrand ME, Holford TR, Bracken M. (2010). Effect of pregnancy on maternal asthma symptoms and medication use. *Obstet Gynecol* 115:559–567.

Bikov A, Pako J, Kovacs D, Tamasi L, Lazar Z, Rigo J, Losonczy G, Horvath I. (2011). Exhaled breath volatile alterations in pregnancy assessed with electronic nose. *Biomarkers* 16:476–484.

Bohács A, Pállinger E, Tamási L, Rigó J Jr, Komlósi Z, Müller V, Dong Y, Magyar P, Falus A, Losonczy G. (2010). Surface markers of lymphocyte activation in pregnant asthmatics. *Inflamm Res* 59:63–70.

Charan NB, Baile EM, Paré PD. (1997). Bronchial vascular congestion and angiogenesis. *Eur Respir J* 10:1173–1180.

Clark DE, Smith SK, He Y, Day KA, Licence DR, Corps AN, Lammoglia R, Charnock-Jones DS. (1998). A vascular endothelial growth factor antagonist is produced by the human placenta and released into the maternal circulation. *Biol Reprod* 59:1540–1548.

Corne J, Chupp G, Lee CG, Homer RJ, Zhu Z, Chen Q, Ma B, Du Y, Roux F, McArdle J, Waxman AB, Elias JA. (2000). IL-13 stimulates vascular endothelial cell growth factor and protects against hyperoxic acute lung injury. *J Clin Invest* 106:783–791.

Dalaveris E, Kerenidi T, Katsabeki-Katsafli A, Kiropoulos T, Tanou K, Gourgoulianis KI, Kostikas K. (2009). VEGF, TNF-alpha and 8-isoprostane levels in exhaled breath condensate and serum of patients with lung cancer. *Lung Cancer* 64:219–225.

Dombrowski MP. (2006). Asthma and pregnancy. *Obstet Gynecol* 108:667–681.

Espinoza J, Uckele JE, Starr RA, Seubert DE, Espinoza AF, Berry SM. (2010). Angiogenic imbalances: the obstetric perspective. *Am J Obstet Gynecol* 203:17.e1–17.e8.

Feltis BN, Wignarajah D, Zheng L, Ward C, Reid D, Harding R, Walters EH. (2006). Increased vascular endothelial growth factor and

receptors: relationship to angiogenesis in asthma. *Am J Respir Crit Care Med* 173:1201–1207.

Font-Ribera L, Kogevinas M, Zock JP, Gómez FP, Barreiro E, Nieuwenhuijsen MJ, Fernandez P, Lourencetti C, Pérez-Olabarría M, Bustamante M, Marcos R, Grimalt JO, Villanueva CM. (2010). Short-term changes in respiratory biomarkers after swimming in a chlorinated pool. *Environ Health Perspect* 118:1538–1544.

Gessner C, Rechner B, Hammerschmidt S, Kuhn H, Hoheisel G, Sack U, Ruschpler P, Wirtz H. (2010). Angiogenic markers in breath condensate identify non-small cell lung cancer. *Lung Cancer* 68:177–184.

Global Initiative for Asthma. Global strategy for asthma management and prevention. Updated 2009. www.ginasthma.com (Accessed 20 August 2011).

Horváth I, Hunt J, Barnes PJ, Alving K, Antczak A, Baraldi E, Becher G, van Beurden WJ, Corradi M, Dekhuijzen R, Dweik RA, Dwyer T, Effros R, Erzurum S, Gaston B, Gessner C, Greening A, Ho LP, Hohlfeld J, Jöbsis Q, Laskowski D, Loukides S, Marlin D, Montuschi P, Olin AC, Redington AE, Reinhold P, van Rensen EL, Rubinstein I, Silkoff P, Toren K, Vass G, Vogelberg C, Wirtz H; ATS/ERS Task Force on Exhaled Breath Condensate. (2005). Exhaled breath condensate: methodological recommendations and unresolved questions. *Eur Respir J* 26:523–548.

Hoshino M, Nakamura Y, Hamid QA. (2001a). Gene expression of vascular endothelial growth factor and its receptors and angiogenesis in bronchial asthma. *J Allergy Clin Immunol* 107:1034–1038.

Hoshino M, Takahashi M, Aoike N. (2001b). Expression of vascular endothelial growth factor, basic fibroblast growth factor, and angiogenin immunoreactivity in asthmatic airways and its relationship to angiogenesis. *J Allergy Clin Immunol* 107:295–301.

Jelkmann W. (2001). Pitfalls in the measurement of circulating vascular endothelial growth factor. *Clin Chem* 47:617–623.

Kanazawa H, Asai K, Hirata K, Yoshikawa J. (2002a). Vascular involvement in exercise-induced airway narrowing in patients with bronchial asthma. *Chest* 122:166–170.

Kanazawa H, Hirata K, Yoshikawa J. (2002b). Involvement of vascular endothelial growth factor in exercise induced bronchoconstriction in asthmatic patients. *Thorax* 57:885–888.

Kastelijn EA, Rijkers GT, Van Moorsel CH, Zanen P, Kwakkel-van Erp JM, Van De Graaf EA, Van Kessel DA, Grutters JC, Van Den Bosch JM. (2010). Systemic and exhaled cytokine and chemokine profiles are associated with the development of bronchiolitis obliterans syndrome. *J Heart Lung Transplant* 29:997–1008.

Kharb S. (2009). Serum markers in pre-eclampsia. *Biomarkers* 14:395–400.

Lee CG, Link H, Baluk P, Homer RJ, Chapoval S, Bhandari V, Kang MJ, Cohn L, Kim YK, McDonald DM, Elias JA. (2004). Vascular endothelial growth factor (VEGF) induces remodeling and enhances TH2-mediated sensitization and inflammation in the lung. *Nat Med* 10:1095–1103.

Lee KY, Lee KS, Park SJ, Kim SR, Min KH, Choe YH, Lee YC. (2008). Clinical significance of plasma and serum vascular endothelial growth factor in asthma. *J Asthma* 45:735–739.

Leung TF, Wong GW, Ko FW, Li CY, Yung E, Lam CW, Fok TF. (2005). Analysis of growth factors and inflammatory cytokines in exhaled breath condensate from asthmatic children. *Int Arch Allergy Immunol* 137:66–72.

Levine RJ, Maynard SE, Qian C, Lim KH, England LJ, Yu KF, Schisterman EF, Thadhani R, Sachs BP, Epstein FH, Sibai BM, Sukhatme VP, Karumanchi SA. (2004). Circulating angiogenic factors and the risk of preeclampsia. *N Engl J Med* 350:672–683.

Maynard SE, Min JY, Merchan J, Lim KH, Li J, Mondal S, Libermann TA, Morgan JP, Sellke FW, Stillman IE, Epstein FH, Sukhatme VP, Karumanchi SA. (2003). Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia. *J Clin Invest* 111:649–658.

Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CP, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J; ATS/ERS Task Force. (2005). Standardisation of spirometry. *Eur Respir J* 26:319–338.

Molvarec A, Ito M, Shima T, Yoneda S, Toldi G, Stenczer B, Vásárhelyi B, Rigó J Jr, Saito S. (2010). Decreased proportion of peripheral blood vascular endothelial growth factor-expressing T and natural killer cells in preeclampsia. *Am J Obstet Gynecol* 203:567.e1–567.e8.

NAEPP expert panel report. (2005). Managing asthma during pregnancy: recommendations for pharmacologic treatment – 2004 update. *J Allergy Clin Immunol* 115:34–46.

Palm M, Basu S, Larsson A, Wernroth L, Åkerud H, Axelsson O. (2011). A longitudinal study of plasma levels of soluble fms-like tyrosine kinase 1 (sFlt1), placental growth factor (PlGF), sFlt1: PlGF ratio and vascular endothelial growth factor (VEGF-A) in normal pregnancy. *Acta Obstet Gynecol Scand* 90:1244–1251.

Park CW, Park JS, Shim SS, Jun JK, Yoon BH, Romero R. (2005). An elevated maternal plasma, but not amniotic fluid, soluble fms-like tyrosine kinase-1 (sFlt-1) at the time of mid-trimester genetic amniocentesis is a risk factor for preeclampsia. *Am J Obstet Gynecol* 193:984–989.

Poulin S, Thompson C, Thivierge M, Véronneau S, McMahon S, Dubois CM, Stankova J, Rola-Pleszczynski M. (2011). Cysteinyl-leukotrienes induce vascular endothelial growth factor production in human monocytes and bronchial smooth muscle cells. *Clin Exp Allergy* 41:204–217.

Rodríguez-Trigo G, Zock JP, Pozo-Rodríguez F, Gómez FP, Monyarch G, Bouso L, Coll MD, Verea H, Antó JM, Fuster C, Barberà JA; SEPAR-Prestige Study Group. (2010). Health changes in fishermen 2 years after clean-up of the Prestige oil spill. *Ann Intern Med* 153:489–498.

Schatz M. (1999). Interrelationships between asthma and pregnancy: a literature review. *J Allergy Clin Immunol* 103:S330–S336.

Tamási L, Bohács A, Pállinger E, Falus A, Rigó J Jr, Müller V, Komlósi Z, Magyar P, Losonczy G. (2005). Increased interferon-γ- and interleukin-4-synthesizing subsets of circulating T lymphocytes in pregnant asthmatics. *Clin Exp Allergy* 35:1197–1203.

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

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

Van De Graaf EA, Out TA, Roos CM, Jansen HM. (1991). Respiratory membrane permeability and bronchial hyperreactivity in patients with stable asthma. Effects of therapy with inhaled steroids. *Am Rev Respir Dis* 143:362–368.