



Periostin in Exhaled Breath Condensate and in Serum of Asthmatic Patients: Relationship to Upper and Lower Airway Disease

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Purpose: Periostin is considered a biomarker for eosinophilic airway inflammation and have been associated with NSAID-Exacerbated Respiratory Disease (NERD) and chronic rhinosinusitis (CRS). In this study, we aimed to evaluate periostin in exhaled breath condensate (EBC) and in serum of patients with various asthma phenotypes. **Methods:** The study included 40 asthmatic patients (22 with NERD) and 17 healthy controls. All the procedures (questionnaire, spirometry, FeNO, nasal swabs, EBC collecting, and blood sampling) were performed on the same day. Periostin concentrations were measured using an ELISA kit. **Results:** Periostin was detected in EBC from 37 of 40 asthmatics and in 16 from 17 of controls. The concentration of periostin in EBC did not differ between the study groups and was not associated with NERD or asthma severity. However, the EBC periostin was significantly higher in asthmatics with CRS as compared to those without (3.1 vs 2 ng/mL, $P=0.046$). Patients with positive bacterial culture from nasal swabs had higher EBC periostin concentrations than those without (3.2 vs 2.1 ng/mL; $P=0.046$). The mean serum periostin level was higher in asthmatics with a 1-year history of exacerbation than in those without (3.2 vs 2.3 ng/mL, $P=0.045$). Asthmatics with skin manifestation of NSAIDs hypersensitivity had higher serum periostin levels as compared to those without (3.5 vs 2.3 ng/mL; $P=0.03$). **Conclusions:** EBC periostin levels seem to reflect intensity of upper airway disease in asthmatics, while serum levels of periostin are associated with asthma activity (exacerbations or FeNO) or NERD subphenotypes.

Key Words: Bronchial asthma; exhaled breath condensate; periostin

INTRODUCTION

Periostin is an extracellular matrix protein, which is produced by a variety of cells, and is involved in cell adhesion and connective tissue regeneration. Its role in the development of cardiovascular, respiratory, allergic, and other inflammatory diseases has been postulated.¹ Based on genome-wide profiling, the periostin gene *POSTN* was found to be one of the most highly expressed genes in bronchial epithelial cells from asthmatics,² and its expression in nasal polyps and chronic rhinosinusitis tissue is also increased.³ Periostin is induced in airway epithelium and lung fibroblasts by IL-4 and IL-13, and seems to be a marker for Th2-inflammation and bronchial and sinonasal remodeling.^{3,4} Serum periostin in severe asthmatics was even a stronger predictor of airway eosinophilia compared to blood eosinophil count, fractional exhaled nitric oxide (FeNO), and total IgE.⁵ On the other hand, it was not possible to distinguish between eosinophilic and non-eosinophilic inflammation in mild to moderate asthmatics based on serum periostin levels.⁶

Elevated serum levels of periostin are associated with the severity of asthma and various asthma phenotypes, *e.g.* late-onset eosinophilic asthma,⁷ and nonsteroidal anti-inflammatory drugs (NSAIDs) exacerbate respiratory disease (NERD).⁸ It has also been documented that periostin may be associated with the severity and chronicity of atopic dermatitis, and possibly other inflammatory skin diseases.⁹

Collecting exhaled breath condensate (EBC) is easy to perform and a non-invasive method of sampling material from the lower airways. Several biomarkers could be found in EBC, including reactive oxygen/nitrogen species, cytokines, and other

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molecules, which are related to the severity of asthma and other respiratory diseases.^{10,11} However, measurements of periostin concentration in EBC have not been reported yet.

We aimed to investigate if periostin could be detected in EBC collected from patients with asthma and healthy subjects. Since measurable concentrations were detected, we investigated possible associations of EBC and serum periostin concentrations with asthma control or severity, and different disease phenotypes including NERD and coexistence of chronic rhinosinusitis.

MATERIALS AND METHODS

Patients

The study included 40 asthmatic patients (22 with NSAIDs-

Exacerbated Respiratory Disease and 18 tolerating NSAIDs) and 17 healthy controls. Asthma was characterized by variable severity (from moderate to severe) and current level of control (only 12.5% patients had controlled asthma according to the Asthma Management and Prevention, Global Initiative for Asthma [GINA] criteria, and 75% of asthmatics had asthma exacerbations during the previous year). All patients were treated with inhaled corticosteroids (ICS) and 7 (17.5%) also received oral CS. The characteristics of the asthmatic group and the comparison of clinical characteristic of NERD and NSAID-tolerant asthmatics are presented in Table. The control group consisted of 17 volunteers (10 males and 7 females; mean age, 51.8±10.54 years, with no history of chronic airway disease or respiratory infection during the previous 4 weeks.

All patients were recruited from our Asthma Clinic and invit-

Table. Clinical characteristics of asthmatic patients in NERD patients (n=20) and NSAIDs-tolerant asthmatics. Comparisons between categorical variables were done with the chi-square test. Quantitative variables were compared using unpaired *t* tests

	Whole asthma group, n=40	NERD, n=22	NSAIDs-tolerant, n=18	NERD vs NSAIDs-tolerant, <i>P</i>	CRS (-) n=16	CRS (+) n=24	CRS (-) vs CRS (+), <i>P</i>
Male, n (%)	9 (22)	3 (16.7)	6 (27.3)	ns	6 (37.5)	3 (12.5)	ns
Mean age (year ± SD)	54.9 ± 10.6	54.9 ± 10.9	54.9 ± 10.7	ns	54.9 ± 13.1	54.9 ± 8.9	ns
Atopy, n (%)	22 (55)	13 (76.5)	9 (47.4)	ns	14 (93.3)	8 (33.3)	<0.001
Hypersensitivity to NSAIDs, n (%)	22 (55)	22 (100)	0	ns	8 (50)	14 (58.3)	ns
FEV1 baseline (% pred; mean ± SD)	73.3 ± 22.1	69.9 ± 25.1	77.4 ± 17.5	ns	70.5 ± 22.5	75.1 ± 22.1	ns
FEV1/FVC baseline (mean ± SD)	68.3 ± 10.6	66.1 ± 11.1	70.9 ± 9.7	ns	67.9 ± 12.4	68.5 ± 9.6	ns
FEV1/FVC postbronchodilator (mean ± SD)	72.8 ± 10.7	70.6 ± 11.2	75.4 ± 10.7	ns	71.2 ± 12.7	73.8 ± 9.4	ns
Patients with fixed airflow limitation; n (%)	14 (35)	9 (40.9)	5 (27.8)	ns	8 (50)	6 (25)	ns
Current treatment							
ICS, n (%)	40 (100)	22 (100)	18 (100)	ns	16 (100)	24 (100)	ns
Low dose*, n (%)	5 (12.5)	1 (4.5)	4 (22.2)	ns	3 (18.75)	2 (8.3)	ns
Medium dose*, n (%)	20 (50)	11 (50)	9 (50)	ns	8 (50)	12 (50)	ns
High dose dose*, n (%)	15 (37.5)	10 (45.5)	5 (27.8)	ns	5 (31.25)	10 (41.7)	ns
LABA, n (%)	39 (97.5)	22 (100)	17 (94.4)	ns	16 (100)	23 (95.8)	ns
Oral steroids, n (%)	7 (17.5)	6 (27.3)	1 (5.6)	ns	2 (12.5)	5 (20.8)	ns
Leukotriene antagonists, n (%)	9 (22.5)	5 (22.7)	4 (22.2)	ns	1 (6.25)	8 (33.3)	0.03
Asthma control and severity							
Controlled*, n (%)	5 (12.5)	3 (13.6)	2 (11.1)	ns	2 (12.5)	3 (12.5)	ns
Partly controlled*, n (%)	10 (25)	4 (18.2)	6 (33.3)	ns	5 (31.25)	5 (20.8)	ns
Uncontrolled*, n (%)	25 (62.5)	15 (68.2)	10 (55.6)	ns	9 (56.25)	16 (66.7)	ns
ACT score (mean ± SD)	15.9 ± 5.4	15.6 ± 5.3	15.7 ± 5.7	ns	15.7 ± 6	16 ± 5.2	ns
Severe asthma [†] , n (%)	8 (20)	7 (31.8)	1 (5.6)	0.029	1 (6.25)	7 (29.2)	ns
Exacerbations last year, n (%)	30 (75)	17 (77.3)	13 (72.23)	ns	11 (68.75)	19 (79.2)	ns
FeNO (ppb; mean ± SD)	36.4 ± 16.6	42 ± 18.5 (18-82)	29.6 ± 11	0.016	33.2 ± 16.4	38.6 ± 16.7	ns
FeNO >50 ppb, n (%)	7 (17.5)	7 (31.8)	0	0.002	1 (6.25)	6 (25)	ns
Positive nasal swab culture	20 (50)	13 (59.1)	7 (38.9)	ns	5 (31.25)	15 (62.5)	ns

*According to GINA¹²; [†]According to ATS criteria¹⁵.

NERD, NSAID-exacerbated respiratory disease; NSAIDs, Nonsteroidal anti-inflammatory drugs; FEV1, forced expiratory volume in 1 second; FEV1/FVC, forced expiratory volume in 1 second % of forced vital capacity; ICS, Inhaled corticosteroids; LABA, long-acting beta-agonists; FeNO, fractional exhaled nitric oxide; CRS, Chronic Rhinosinusitis.

ed to a single visit, during which all the procedures (questionnaire, spirometry, FeNO measurement, skin prick tests, EBC collecting, and blood sampling) were performed. The questionnaire included questions about family history and environmental factors, including smoking, concomitant chronic diseases, allergic diseases, respiratory symptoms, medication load, and use of health services. The study was approved by the Bioethics Committee of the Medical University of Lodz, and all patients signed an informed consent.

Definitions

Asthma was diagnosed according to the Global Strategy for the GINA 2011 criteria.¹² NERD was diagnosed based on clinical history, including at least 2 episodes of asthma exacerbation associated with NSAIDs intake and/or ASA oral challenge.¹³ Asthma exacerbation was defined as the presence of at least one of the following events during the past 12 months: an unscheduled visit to the doctor's office, a course of oral corticosteroids, hospitalization or emergency service intervention due to worsening of asthma-related symptoms. The level of asthma control was established according to the GINA 2011 guidelines¹² and assessed by the Asthma Control Test¹⁴ Severe/difficult to control asthma was defined according to the ATS Workshop 2000 criteria.¹⁵ Chronic rhinosinusitis was diagnosed according to the EPOS criteria (typical clinical symptoms, confirmed by rhinoscopy, and sinus imaging if necessary).¹⁶

Skin prick tests (SPTs)

The panel of skin prick tests (Allergopharma, Reinbek, Germany) included the following allergens: *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat, dog, tree mix, grass mix, weed mix, *Alternaria tenuis*, and *Cladosporium herbarum*. A positive result was defined as a wheal of ≥ 3 mm in diameter. Atopy was diagnosed in the presence of at least 1 positive skin test.

Spirometry

Spirometry was performed according to ERS standards,¹⁷ using a Lungtest 500 spirometer (MES, Kraków, Poland). Before performing spirometry, patients were asked to discontinue long-acting beta-agonists (LABA), teophylline, and leukotriene receptor antagonist for 24 hours, short-acting β -agonist (SABA) and ipratropium bromide for 6 hours. A reversibility test after salbutamol inhalation (400 mcg salbutamol MDI) was performed in all asthmatic patients.

FeNO measurement

Patients underwent online single breath maneuvers according to ATS/ERS guidelines¹⁸ using the HypAir FeNO (Medisoft, Belgium). The mean value of at least 2 successful measurements was analyzed. FeNO measurement was performed before spirometry and EBC collecting.

Collection of EBC

EBC samples were collected using TURBODECCS 09 unit (Medivac, Parma, Italy) according to the manufacturer's instructions and ERS/ATS recommendations.¹⁹ Patients were asked to breath tidally for 10 minutes through the mouthpiece with a saliva trap in the sitting position. The temperature of the condenser was set at -10°C . The respiratory samples were immediately stored at -80°C and kept frozen until analysis. The collection of EBC was performed before spirometry and after FeNO measurement.

Nasopharyngeal secretions and bacterial cultures

Nasopharyngeal samples for the detection of bacteria were collected using swabs flocced with soft nylon fiber (Copan, Italy). The swabs were transported to the Microbiology Laboratory in Amies transport medium and inoculated on selective and non-selective media no longer than 2 hours after collection. The plates were evaluated 24 and 48 hours after inoculation for the presence of *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Staphylococcus aureus*, and *Streptococcus pyogenes*.

Periostin measurements in EBC and serum

Periostin was measured in the sera and in the EBC using an enzyme-linked immunoassay (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

Statistical analysis

Categorical variables were compared using the chi-square test. Quantitative variables are presented as means and standard deviation and compared using unpaired *t*-tests. Continuous variables were compared using Pearson's correlation. The statistical analysis was performed using Statistica (StatSoft, Tulsa, OK, USA). *P* values < 0.05 were accepted as statistically significant.

RESULTS

Detectable concentrations of periostin were found in EBC samples from 37/40 asthmatics and in 16/17 healthy subjects. Periostin was detected in the sera of all study participants. The mean periostin level was lower in EBC samples than in serum (2.9 ± 1.7 vs 24.6 ± 11.3 ng/mL; $P < 0.001$). There were no differences in EBC or serum periostin concentrations between asthma patients and control subjects (Fig. 1). No correlation between EBC and serum periostin concentrations was found in the entire group of asthmatic patients or in the control group.

Periostin in EBC

Periostin concentrations in EBC were not related to asthma severity, asthma control, intensity of ICS treatment, lung function, or presence of fixed airflow limitation defined as lack of re-

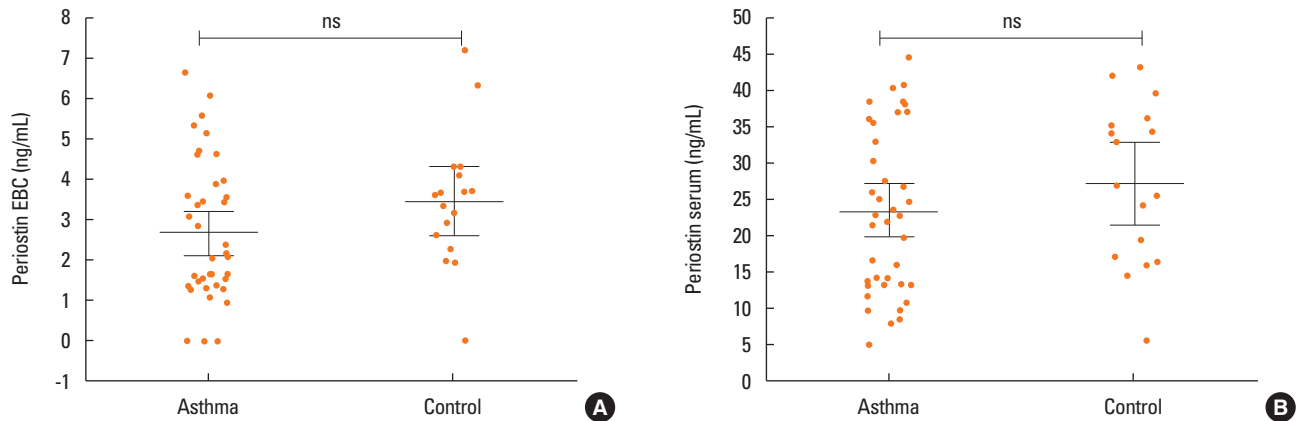


Fig. 1. Periostin concentrations (ng/mL) in EBC (A) and serum (B) of asthmatics, and healthy controls (differences are not statistically significant). Data are presented as means and 95% confidence intervals.

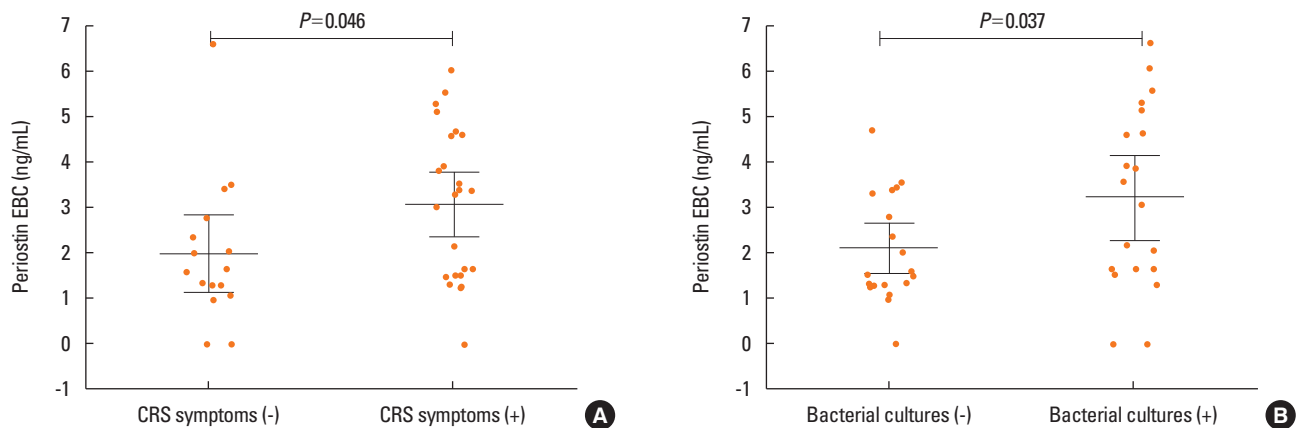


Fig. 2. Periostin concentration (ng/mL) in EBC (A) from asthmatics with and without chronic rhinosinusitis symptoms in the previous 3 months and (B) from asthmatics with and without positive bacterial cultures from nasal swabs. Data are presented as means and 95% confidence intervals.

versibility to salbutamol (data not shown). There was no relationship between periostin levels in EBC and age/sex/presence of atopy.

However, in asthmatic patients with diagnosis of chronic rhinosinusitis (CRS), who reported sinonasal symptoms during the previous 3 months ($n=24$), periostin levels in EBC were significantly higher in subjects without CRS symptoms than in those without (3.1 ± 1.7 vs 2 ± 1.6 ng/mL, $P=0.046$) (Fig. 2A).

Twenty of 40 patients with asthma had positive bacterial cultures from nasal swabs and the most common bacteria were *Staphylococcus aureus* ($n=14$) followed by *Streptococcus pneumoniae* ($n=5$), *Haemophilus influenzae* ($n=5$), *Haemophilus parainfluenzae* ($n=5$), and *Streptococcus pyogenes* ($n=5$). Asthmatic patients with positive bacterial cultures from nasal swabs had higher levels of periostin in EBC than patients with negative cultures (3.2 ± 2 vs 2.1 ± 1.2 ng/mL; $P=0.037$) (Fig. 2B).

Serum periostin levels

The mean serum periostin concentrations were significantly el-

evated in asthmatics who reported an exacerbation and/or required unscheduled doctor office visit within the previous 12 months, as compared to those without (27 ± 11.5 vs 19.5 ± 10.1 ng/mL, $P=0.036$). Patients treated with leukotriene antagonist added to ICS had a significantly lower serum periostin level (16.2 ± 10.5 vs 25.6 ± 10.9 ng/mL, $P=0.028$). A positive correlation between serum periostin and FeNO levels ($r=0.33$; $P=0.039$) was found, and asthmatics with a very high FeNO level (over 50 ppb) had a higher serum periostin level than subjects with a lower FeNO (35.1 ± 4.4 vs 21 ± 10.9 ng/mL; $P=0.002$) (Fig. 3A).

For all groups of asthmatics, there was no relationship between periostin levels in serum and age/sex/presence of atopy/asthma severity, intensity of ICS treatment, lung function/airway reversibility after bronchodilator nebulization.

Mean periostin concentrations in serum were similar in NERD patients and NSAIDs-tolerant asthmatics (26.5 ± 10.7 vs 19.7 ± 11.5 ng/mL; ns). However, NERD patients with history of asthma exacerbation within last 12 months ($n=17$) had significantly higher mean serum periostin level as compared to those with-

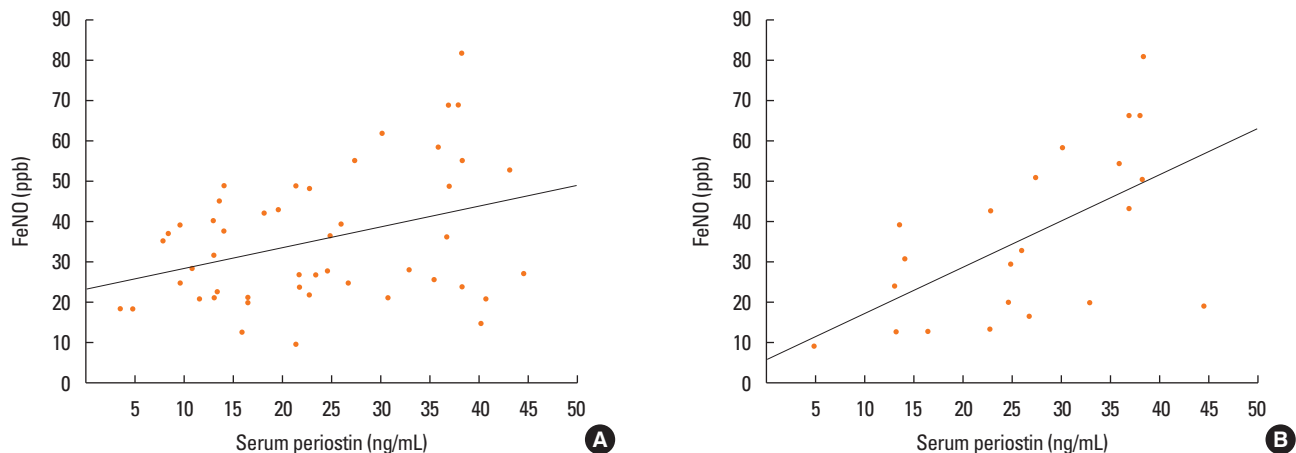


Fig. 3. Correlation between levels of periostin and FeNO in serum (A) in the entire group of asthmatics ($r=0.33$; $P=0.039$) and between (B) levels of FeNO in exhaled breath in patients with NERD ($r=0.58$; $P<0.004$).

out ($n=5$), (29.4 ± 8.5 vs 16.5 ± 12.1 ng/mL; $P=0.013$). There was also a stronger correlation between serum periostin concentration and FeNO level in all groups ($r=0.59$; $P<0.004$) (Fig. 3B).

NERD patients, who reported skin symptoms (urticaria and/or angioedema) after NSAIDs intake in addition to bronchial obstruction ($n=16$), had higher levels of serum periostin than those with only bronchial symptoms after NSAIDs and NSAIDs-tolerant asthmatics ($n=24$), (29.2 ± 9.7 vs 19.6 ± 11 ng/mL; $P=0.007$).

DISCUSSION

This is the first study reporting detectable concentrations of periostin in EBCs—biological material that is considered to originate from lower airways. Concentrations of periostin were similar in EBC, as well as in serum, collected from patients with asthma and healthy individuals, which is difficult to explain because in previous studies increased levels of this molecule have been found in induced sputum and were associated with eosinophilic airway inflammation.²⁰ It seems likely that EBC and serum levels of periostin in our asthma patients were affected by chronic asthma treatment. All patients received inhaled corticosteroids and majority of them (87.5%) were on medium or high doses of ICS. It has previously been shown that steroid-naïve patients had a 10% reduction in serum periostin level after a 48-week treatment with ICS²¹ and periostin gene (POSTN) expression in the airway epithelium is decreased following ICS therapy.² In addition, 17% of our asthma patients were treated with systemic corticosteroids, which may have reduced periostin concentration in the serum of asthmatic patients.²² We also observed that 9 patients treated with leukotriene antagonist added to ICS had a significantly lower serum periostin level than those treated with ICS only, which suggests that the intensity and/or the type of anti-inflammatory treatment may influence serum concentrations of periostin.

The EBC periostin level was not correlated with parameters of asthma control and severity, and lung function or FeNO levels, suggesting that it may not reflect lower airway inflammation. This is in contrast to studies assessing periostin in induced sputum, which demonstrated the association of periostin levels with asthma severity or the presence of fixed bronchial obstruction as well as with the percentage of eosinophils in the blood and sputum.^{20,23} However, our asthmatic patients with CRS who reported symptoms during the previous 3 months had on average 50% higher levels of periostin in EBC than those without. Patients with and without CRS symptoms were similar with respect to all clinical/pathophysiological features of asthma, including severity, asthma control, or spirometry. These observations strongly point at differences in upper airway inflammation type or intensity as the source of differences in EBC periostin levels. Although EBC is considered to sample primarily lower airways, it may also contain proteins and other particles derived from the upper respiratory tract.¹⁰ Using EBC collection set with a saliva trap, a 1-way valve, and a nasal clip, we attempted to avoid sampling from the upper airways, but contamination with the upper airway fluid could not be completely avoided. Thus, it is likely that at least part of periostin in EBC originated from inflamed upper airways. Periostin was previously detected in nasal lavage fluids, and an increased expression of periostin in sinonasal mucosa was observed in patients with CRS and polyps or with allergic rhinitis³ which indicated that upper airways may be a substantial source of this mediator, and this molecule has been implicated in the pathogenesis of CRS.^{3,24,25} These observations are also in line with previously reported increased periostin levels in the sera of asthmatics with concomitant nasal disorders.²⁶ Interestingly, patients with positive bacterial cultures from nasopharyngeal secretions had higher levels of periostin in EBC than those without. Bacterial infections may exacerbate CRS, and host-microbial interactions seem to be important for the pathogenesis of the sino-nasal disease.²⁷

Our findings suggest that the presence of bacteria in the upper airways, even without evident symptoms of acute infections, may increase local production and/or release of periostin, which is in turn detected in the EBC. Thus, it appears that periostin in EBC may originate from both lower and upper airways, which may explain lack of correlations with FeNO or asthma severity/control.

In contrast to EBC, a positive correlation between serum periostin and FeNO was observed, and higher mean periostin serum concentrations in patients with very high (above 50 ppb) FeNO level were detected. These observations are in line with those of previous reports documenting association of periostin levels in serum with FeNO levels⁶ or with blood and sputum eosinophilia²⁰ in asthmatic patients. The serum periostin level has been even proposed as a surrogate marker for Th2-inflammation driven by IL 13, and the serum periostin level has been proposed as a better marker for airway eosinophilia than blood eosinophilia.⁵ Furthermore, our patients with a history of asthma exacerbation in the past 12 months had higher levels of periostin than those without. Similarly, Scichilone *et al.*²⁸ found that serum periostin level was a predictor of asthma exacerbation at a 1-year follow-up.

EBC concentrations of periostin were significantly lower as compared to serum levels, which is in line with previous studies reporting lower periostin levels in induced sputum^{20,23} and bronchial lavage fluid (BLF)²⁹ than in the serum. In the studies analyzing induced sputum, the concentration of periostin was above the lower limit of detection of the immunoassays used,^{20,23} and periostin could be detected in BLF only in the samples with 10-fold concentration.²⁹ Most of our study subjects (37/40 of asthmatics and 16/17 controls) had EBC periostin levels above the lower detection limit of the immunoassay used. We did not find any correlation between serum and EBC periostin concentrations. Similarly, Simpson *et al.*²³ reported no associations between serum and IS periostin levels.

NERD represents asthma phenotype with higher than average disease severity, complicated by CRS with nasal polyps and characterized by severe eosinophilic inflammation in both upper and lower airways.^{30,31} It has been reported that serum periostin level is associated with NERD phenotype, and correlates with blood eosinophil counts and sputum eosinophilia.⁸ In our group, more NERD patients had severe asthma as compared to NSAIDs-tolerant asthmatics (31.8% vs 5.6%), and average NERD patients had higher mean FeNO, reflecting ongoing more intense bronchial inflammation. Although NERD patients from our study group had only insignificantly higher serum periostin concentrations as compared to NSAIDs-tolerant asthmatics, those with a history of asthma exacerbation within the last 12 months had significantly higher mean serum periostin levels as compared to those without. Furthermore, NERD patients, who in addition to respiratory symptoms developed skin manifestations following NSAIDs, on average showed sig-

nificantly higher serum periostin levels. Periostin has been shown to be highly expressed in the skin and serum periostin levels were associated with inflammatory skin diseases.⁹ Thus, our observations suggest a possible association of periostin with a subtype of NERD phenotype, further pointing at pathophysiological heterogeneity of this group of asthmatics.³⁰

In summary, we demonstrated that periostin was detectable in EBC from both asthma patients and healthy subjects, but periostin EBC concentrations did not show any correlation with asthma parameters. However, increased periostin levels in EBC were associated with symptoms of CRS and positive nasopharyngeal bacterial cultures, which can suggest that inflamed upper airways may be a source of periostin. In contrast, periostin levels in serum were associated with some asthma activity markers (history of exacerbations or FeNO) or NERD subphenotypes, indicating that the source of the biological material, in which periostin is measured, may determine the utility of periostin as a biomarker for asthma.

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