[Original Research]

CHEST

Looking for Airways Periostin in Severe Asthma

Could It Be Useful for Clustering Type 2 Endotype?

Giovanna E. Carpagnano, MD, PhD; Giulia Scioscia, MD; Donato Lacedonia, MD, PhD; Piera Soccio, MSc; Giorgia Lepore, MD; Marina Saetta, MD, FCCP; Maria Pia Foschino Barbaro, MD; and Peter J. Barnes, MD, DSc, FCCP

BACKGROUND: Severe asthma is heterogeneous clinically and biologically and is often difficult to control. In particular, the type 2 (T2) immunity endotype of severe asthma is gaining increasing interest because it is susceptible to newly developed biologic treatments that can transform the quality of life of these patients. The aim of this study was to analyze periostin concentrations in the airways of patients with severe asthma, evaluating its role in clustering the T2 endotype.

METHODS: We enrolled 40 consecutive patients with severe asthma (T2 endotype: n = 25; non-T2 endotype: n = 15), 21 patients with mild to moderate asthma, and 15 healthy control subjects. All subjects enrolled underwent exhaled breath condensate (EBC) and sputum collection, eosinophil count in blood, fractional exhaled nitric oxide, and IgE measurement. Periostin was assessed by an enzyme-linked immunosorbent assay kit on EBC and induced sputum (IS) supernatant.

RESULTS: We were able to detect higher periostin levels in the EBC (0.75 ± 0.46 vs 0.70 ± 0.19 vs 0.11 ± 0.05 ng/mL, $P < .05$ and $P < .01$) and in IS (0.55 ± 0.23 vs 0.31 ± 0.13 vs 0.16 ± 0.120 ng/mL, $P < .05$ and $P < .01$) of patients with severe asthma compared with patients with mild to moderate asthma and healthy control subjects, respectively. We further found an increase of periostin levels in both samples in T2 endotype compared with non-T2 endotype (EBC: 0.88 ± 0.46 vs 0.52 ± 0.46 ng/mL; IS: 0.69 ± 0.19 vs 0.39 ± 0.16 ng/mL; $P < .05$) and a correlation between periostin levels in EBC and sputum.

CONCLUSIONS: We found that periostin is measurable in the airways and increased in patients with severe asthma, especially in those from the T2 endotype. Unlike serum periostin, which may be derived from several sources outside the lung, airways periostin is a useful marker of severe eosinophilic asthma and may help to phenotype patients that will respond to the biologic agents.

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KEY WORDS: asthma; exhaled breath condensate; induced sputum; inflammation; periostin

ABBREVIATIONS: EBC = exhaled breath condensate; FENO = fractional exhaled nitric oxide; IS = induced sputum; NO = nitrogen oxide; T2 = type 2

AFFILIATIONS: From the Institute of Respiratory Diseases (Drs Carpagnano, Scioscia, Lacedonia, Lepore, and Barbaro; and Ms Soccio), Department of Medical and Surgical Sciences, University of Foggia, Foggia, Italy; Department of Cardiac, Thoracic, and Vascular Sciences (Dr Saetta), University of Padova, Padova, Italy; and the Airway Disease Section (Dr Barnes), National Heart and Lung Institute, Imperial College London, London, England.

Drs Scioscia and Lacedonia contributed equally to this manuscript.

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CORRESPONDENCE TO: Maria Pia Foschino Barbaro, MD, Department of Medical and Surgical Sciences, Institute of Respiratory Diseases, University of Foggia – Italy, 71100 - Foggia, viale degli Aviatori, Foggia, Italy; e-mail: mariapia.foschino@unifg.it

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Advancements in molecular phenotyping reveal heterogeneity within patients with asthma, with multiple endotypes[1] whose varying expression depends on the interplay between numerous environmental factors and the inheritance of a broad range of susceptibility genes.

The type 2 (T2) immunity severe asthma endotype is one of the most consistent endotypes to emerge, probably because it is a key driver in nearly one-half of all patients with asthma.[1,2]

Its identification today is considered important for improving the management of patients with symptoms that are not controllable with conventional inhalers.[3] This justifies the efforts of this last decade to develop noninvasive biomarkers for this endotype that are characterized by eosinophilic airway inflammation.[3] Biomarkers could help to stratify asthma into T2 endotype, to predict future risk, and to target T2-oriented therapies to patients that are likely to respond.[4]

Several markers have been proposed for the T2 endotype, such as eosinophil count in blood, eosinophil count in sputum, fractional exhaled nitric oxide (FENO), and serum periostin level. However, a single-marker approach may not be successful; therefore, a combination approach is more likely to be useful. The association of eosinophil count in blood, serum periostin level, and FENO previously proved to be successful in predicting a better response to biologic therapy with the anti-IgE antibody omalizumab.[5] In addition, in the subanalysis of the Kinki Hokuriku Airway disease Conference, the potential utility of composite biomarkers, such as FENO and serum periostin levels, was used to stratify patients into four groups that also reflected severity of asthma in terms of exacerbations.[3]

Periostin is an extracellular matrix protein that plays an important role in the T2 endotype of asthma, and serum concentrations have been found to be the most significant predictor of eosinophilic airway inflammation in asthma followed by FENO, blood eosinophils, and IgE.[6]

Previous studies have indicated that adult patients with high levels of serum periostin show a better response to treatment with inhaled corticosteroids,[7] omalizumab, or lebrikizumab (anti-IL-13) and IL-4Ra,[4] suggesting that periostin is linked to increased inflammatory or immunologic activity. This has led to the consideration of periostin as a predictive biomarker to identify patients most likely to respond to such monoclonal antibody therapies. Serum levels of periostin previously exhibited very low variability and high reproducibility.[8]

Airway epithelial cells have a high expression of periostin; therefore, measuring this protein in the airways may give more specific information about eosinophilic inflammation in the airways, whereas serum periostin may reflect periostin derived from additional sources, such as the skin and the gastrointestinal tract.[9] Furthermore, periostin has shown an upregulated response to stress/challenge stimuli, regardless of physiology or disease, and to measure it in serum could represent a disadvantage because several common factors may affect it, such as exercise and skin injury.[10]

To better explore the potential of airway periostin, BAL, induced sputum (IS), and recently the exhaled breath condensate (EBC) have been studied.[11]

In view of the simplicity and noninvasive nature of EBC collection, we think that periostin might prove to be a useful biomarker of T2 airway inflammation.

The aim of our study was to analyze the periostin concentrations in the airways of patients with severe asthma, evaluating its role in the T2 endotype classification and comparing it with other biomarkers.

Materials and Methods

Patients

We screened 98 consecutive patients (60 with severe asthma and 38 with mild to moderate asthma) from the outpatient facility of the Institute of Respiratory Disease, University of Foggia, Foggia, Italy, and finally enrolled 61 patients: 40 with severe asthma (11 men) and 21 with mild to moderate asthma (12 men). We also enrolled 15 healthy age-matched subjects (8 men) as control subjects (Table 1). The main reasons for exclusion of the screened subjects are as follows: (1) randomization in a controlled trial, (2) treatment with biologic therapy, (3) no release of informed consent, or (4) an upper respiratory tract infection during the last 4 weeks.

We divided patients with severe asthma into two groups according to eosinophil count in blood and sputum samples: 25 were included in the T2 endotype group (elevated sputum level $>$ 3% and/or blood eosinophil count $\geq$ 400 cells/μL on at least two occasions) and 15 were included in the non-T2 endotype group (elevated neutrophil level $\geq$ 40% but blood eosinophil count $<$ 300 cells/μL on at least two occasions) (Table 2).

Written informed consent was obtained from all subjects, and the institutional ethics committee of the University of Foggia approved the study (institutional review board approval No. 17/CE/2014). Patients with asthma were classified and treated according to GINA guidelines.[12] All patients with asthma were assessed at a period of stability and at least 4 weeks after an upper respiratory tract infection.

At the first visit, a complete baseline questionnaire requesting information on medical history was administered to all subjects.

TABLE 1] Demographic, Clinical, and Functional Characteristics of the Study Population

Characteristics	Control Subjects (n = 15)	SA Group (n = 40)	MMA Group (n = 21)
Demographic and clinical			
Females	7 (46.6)[a]	29 (72.5)[a, b]	9 (42.8)[b]
Age, y	62 ± 5.45	58 ± 11	53 ± 18
Smokers	5 (33.3)[a]	18 (45)[a, b]	5 (23.8)[b]
Pack-years	5 ± 4[a, c]	9 ± 8[a]	8 ± 5[c]
BMI, kg/m^2	25 ± 5	28 ± 5	26 ± 5
Age of onset, y	…	40 ± 15[b]	23 ± 8[b]
Exacerbations per year	…	2 ± 1[b]	1 ± 1[b]
Atopy	…	27 (67.5)[b]	10 (47.6)[b]
ACT	…	16 ± 4[b]	20 ± 3[b]
ACQ	…	3 ± 2[b]	1 ± 0.7[b]
Lung function			
FEV_1, pre-BD, % predicted	85 ± 5[a]	67 ± 9[a, b]	78 ± 14[b]
FEV_1/FVC, pre-BD, %	82 ± 12[a, c]	62 ± 12[a]	68 ± 10[c]
Reversibility, %	5 ± 4[a, c]	11 ± 6[a]	13 ± 9[c]
TLC, % predicted	104 ± 13	96 ± 14	98 ± 14
VR, % predicted	89 ± 20	110 ± 26	97 ± 25
Pharmacologic treatment	…	High dose of ICS/LABA + tiotropium	ICS and low to medium dose ICS/LABA
Biomarkers			
$FENO_{50}$, ppb	15 ± 6[a, c]	40 ± 21[a, b]	22 ± 18[b, c]
Blood eosinophil count, %	2 ± 1[a, c]	6 ± 4 [a]	4 ± 2[c]
Blood neutrophil level, %	34 ± 13[a, c]	61 ± 16 [a, b]	49 ± 12[b, c]
Eosinophil IS count, %	1 ± 1[a, c]	7 ± 2[a, b]	5 ± 4[b, c]
Neutrophil IS level, %	38 ± 18[a, b]	48 ± 15[a, b]	37 ± 12[b]

Values are mean ± SD or No. (%). ACT = Asthma Control Test; ACQ = Asthma Control Questionnaire; BD = bronchodilator; $FENO_{50}$ = fractional exhaled nitric oxide at a flow rate of 50 mL/s; $FENO_{350}$ = fractional exhaled nitric oxide at a flow rate of 350 mL/s; ICS = inhaled corticosteroids; IS = induced sputum; LABA = long-acting β2 adrenergic receptor agonists; MMA = mild to moderate asthma; SA = severe asthma; TLC = total lung capacity; VR = residual volume.

[a]$P < .05$ between control subjects and patients with SA.

[b]$P < .05$ between patients with SA and patients with MMA.

[c]$P < .05$ between control subjects and patients with MMA.

They were then given a physical examination, atopy assessment, and spirometry with bronchodilator reversibility test. During the second visit, subjects underwent EBC collection, blood analysis for eosinophil count, FENO measurement, and finally sputum induction.

None of the patients enrolled were included in a randomized controlled trial or treated with biologic therapy.

We classified patients as T2 and non-T2 based on clinical and biologic criteria.

TABLE 2] Patients With Severe Asthma Endotypes

Markers	T2 (n =25)	Non-T2 (n =15)
$FENO_{50}$	35 ± 18[a]	18 ± 7[a]
Blood eosinophil count, cells/μL	0.45 ± 0.28[a]	0.19 ± 0.05[a]
Blood neutrophil level, cells/μL	3.8 ± 0.32[a]	5.6 ± 2.7[a]
Eosinophil IS count, % total cells	7 ± 2[a]	3 ± 2[a]
Neutrophils IS level, % total cells	38 ± 18[a]	48 ± 15[a]

Values are mean ± SD. T2 = type 2. See Table 1 legend for expansion of other abbreviations.

[a]$P < .05$ between patients with T2 SA and patients with non-T2 SA.

Atopic Status

The skin prick test was performed for a panel of inhalant allergens as previously described for common aeroallergens (Lofarma).

Lung Function

Pulmonary function tests were performed. FEV_1, FVC, and plethysmographic lung volume were measured using a spirometer (Sensormedics) following international standards in all subjects.[13,14] The best value of three maneuvers was expressed as a percentage of the predicted normal value. After baseline evaluation, spirometry was repeated 15 min after the subjects had inhaled 400 µg of salbutamol. Reversibility of airway obstruction was expressed in terms of the percent changes from baseline of FEV_1.

IS Collection and Processing

According to the method described by Toungoussova et al,[15] sputum was induced through inhalation of hypertonic saline solution (4.5%) with an ultrasonic nebulizer (DeVilbiss 65; DeVilbiss Corporation) and analyzed after selection of mucus plugs. In patients with severe asthma, we used spontaneous sputum or the sputum produced after inhalation of isotonic saline solution when they were particularly uncontrolled.

Nine healthy subjects and 10 patients with severe asthma were not able to produce adequate sputum samples (defined as containing at least 500 nonsquamous cells), and their samples were discarded. The sputum (spontaneous or induced) was used for cytologic analysis and the supernatant was used for periostin analysis.

EBC Collection and Processing

Then 2 mL of EBC was collected in one setting from each patient at the time of diagnosis. EBC was collected using a condenser (EcoScreen; Jaeger). The subjects breathed through a mouthpiece and a two-way non-rebreathing valve that also served as a saliva trap. They were asked to breathe at a normal frequency and tidal volume, wearing a nose clip, for a period of 10 min. If the subjects felt saliva in their mouth, they were instructed to swallow it. Condensate, at least 1 mL, was collected and stored at 4°C, transferred to Eppendorf tubes (Eppendorf Italia), and immediately stored at −70°C. Samples were analyzed within 3 months of collection. To exclude saliva contamination, the amylase activity was analyzed in EBC.

Periostin Analysis

Initially, a proprietary sandwich enzyme-linked immunosorbent assay (ELISA) (Human Periostin ELISA Kit; Thermo-Fisher Scientific) was used to determine periostin levels in undiluted samples of EBC and sputum supernatants. The intra-assay variability was < 10% and the interassay variability was < 12%, with a detection limit of 0.08 ng/mL. Because we achieved unexpected results, below the detection limit, we chose to measure periostin levels in undiluted samples of EBC and sputum supernatants using a specific enzyme immunoassay kit (DuoSet ELISA; R&D Systems) according to the manufacturer's recommendations because this is a more sensitive assay.

Prior to the test, the EBC was concentrated with Microcon-30kDa Centrifugal Filter Unit with Ultracel-30 membrane (Merk Millipore) and then analyzed together with nonconcentrated EBC samples. We carried out several experiments with both EBC samples (concentrated and not), and then we compared the results. Our results showed no significant difference in the concentrations of periostin; therefore, we decided to use the unprocessed EBC samples for the next test.

Measurement of FENO

A rapid-response chemiluminescence nitrogen oxide (NO) analyzer (NIOX MINO; Aerocrine) was used to quantify NO. Two-point calibrations were performed daily using 5.2 parts per million calibration gas. FENO was measured using a previously described restricted breath technique, which used expiratory resistance and positive mouth pressure to close the velum and exclude nasal NO, and a constant expiratory flow of 50 mL/s. Repeated exhalations were performed until three plateaus agreed within 5%.

Statistical Analysis

To assess the difference between the periostin levels in the EBC and the supernatant of the IS of different groups (patients with severe asthma vs patients with mild to moderate asthma vs control subjects, T2 vs non-T2 endotypes), we performed an analysis of variance and a *t* test considering $P < .05$ as statistically significant. Correlations were assessed using Spearman ρ test.

Results

Anthropometric, clinical, functional, and biologic data of subjects enrolled are reported in Tables 1 and 2.

We found that periostin was detectable in all EBC samples collected. We found a significantly higher concentration of periostin in EBC of patients with severe asthma than in patients with mild to moderate asthma and healthy control subjects (0.75 ± 0.46 vs 0.70 ± 0.19 vs 0.11 ± 0.05 ng/mL, respectively; $P < .05$ and $P < .01$) (Fig 1). Particularly higher exhaled periostin levels were found in T2 endotype than in non-T2 endotype (0.88 ± 0.46 vs 0.52 ± 0.46 ng/mL, respectively; $P < .05$) (Fig 2). Significantly higher levels of EBC periostin were found in women with severe asthma compared with men with severe asthma (0.86 ± 0.46 vs 0.44 ± 0.27 ng/mL, respectively; $P < .05$) and in patients with severe atopic asthma compared with patients who were nonatopic (0.85 ± 0.44 vs 0.57 ± 0.39 ng/mL, respectively; $P < .05$).

We found that periostin was also measurable in all IS supernatant samples collected: 30 patients with severe asthma, 21 patients with mild to moderate asthma, and 6 healthy control subjects. Similar results were found in the IS; that is, significantly higher levels of periostin were found in patients with severe asthma compared with patients with mild to moderate asthma and control subjects (0.55 ± 0.23 vs 0.31 ± 0.13 vs 0.16 ± 0.120 ng/mL, respectively; $P < .05$ and $P < .01$) (Fig 3). In particular, there were significantly higher levels in T2 endotype than in non-T2 endotype (0.69 ± 0.19 vs 0.39 ± 0.16 ng/mL, respectively; $P < .05$) (Fig 4).

We found a significant correlation between EBC and IS periostin in patients with severe asthma ($\rho = 0.44$, $P < .05$) (Fig 5). We did not find any correlations between airway periostin levels and blood or sputum eosinophil counts, FENO levels, total IgE levels, BMI, or Asthma Control Test scores.

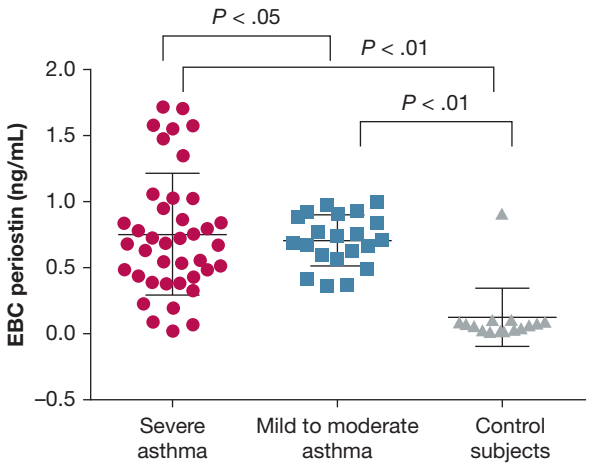

Figure 1 – *EBC periostin (ng/mL) of patients with severe asthma, patients with mild to moderate asthma, and control subjects. EBC = exhaled breath condensate.*

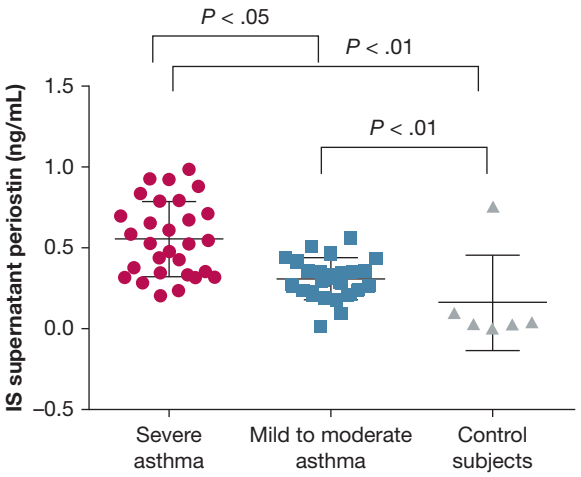

Figure 3 – *IS supernatant periostin (ng/mL) of patients with severe asthma, patients with mild to moderate asthma, and control subjects. IS = induced sputum.*

Discussion

In this study, we have shown that periostin levels are detectable in EBC and in IS supernatant with significant differences between patients with severe asthma and patients with mild to moderate asthma and healthy control subjects. Furthermore, we have described the highest concentrations of airway periostin in patients with severe asthma of T2 endotype, particularly in female patients and patients with atopic severe asthma. Finally, we have found a significant correlation in periostin concentrations between the investigated airways samples in patients with severe asthma.

Previously, Górska et al[16] showed that periostin is detectable in serum, IS, EBC, and BAL fluid in patients with mild to moderate asthma, mild to moderate COPD, and control subjects. Wardzyńska et al[9] evaluated periostin in the EBC and serum of various asthma phenotypes: patients who were nonsteroidal antiinflammatory drug tolerant, patients with nonsteroidal antiinflammatory drug-exacerbated respiratory disease, patients who were chronic rhinosinusitis-positive, and patients who were chronic rhinosinusitis-negative.

To our knowledge, this is the only study to date where periostin levels have been measured in the airways (EBC and IS) of patients with severe asthma, subdivided into the two main endotypes (T2 and non-T2 endotypes). We also compared airway periostin levels in patients with severe asthma with patients with mild to moderate asthma and healthy control subjects. Higher periostin

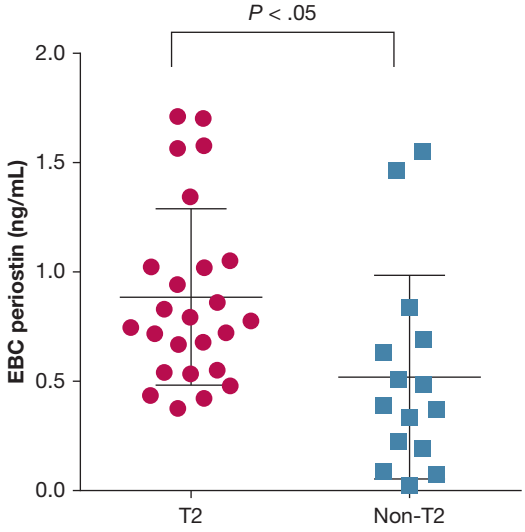

Figure 2 – *EBC periostin (ng/mL) of patients with severe asthma of T2 group and non-T2 group. See Figure 1 legend for expansion of abbreviation.*

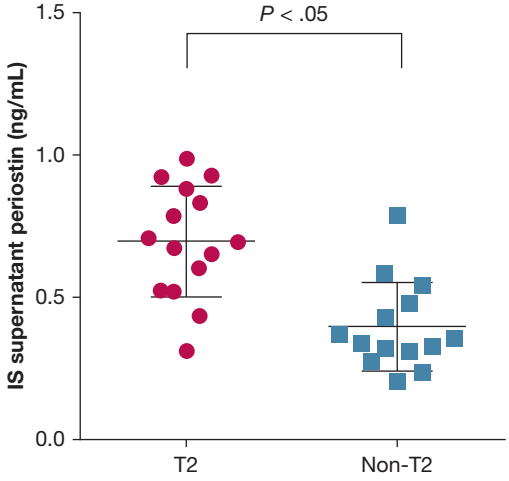

Figure 4 – *IS supernatant periostin (ng/mL) of patients with severe asthma of T2 group and non-T2 group. See Figure 3 legend for expansion of abbreviation.*

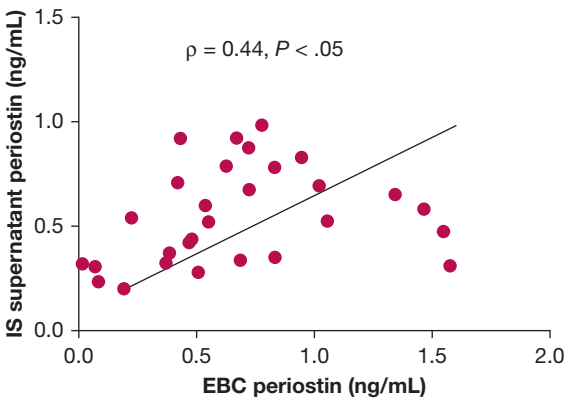

Figure 5 – *Correlation between EBC periostin (ng/mL) and IS supernatant periostin (ng/mL) of patients with severe asthma (ρ = 0.44, P < .05). See Figure 1 and 3 legends for expansion of abbreviations.*

concentrations were found in both EBC and IS of patients with severe asthma of T2 endotype. This is in good agreement with results reported by Górska et al,[16] who described increased periostin levels in eosinophilic asthma, which supports that periostin detected in airways could be more specific in the endotyping of subjects compared with periostin in other biologic fluids, such as blood. In the past, periostin in serum had been considered stable with a small coefficient of variation[9] in contrast with FENO and blood and sputum eosinophil counts, which suggested that high serum periostin levels might imply a more static disease process, whereas FENO and eosinophil counts could reflect more dynamic disease activity, such as during exacerbations of asthma or loss of asthma control.[3] The higher periostin levels that we described in EBC and IS supernatant of patients with severe asthma compared with patients with mild to moderate asthma, and the lack of differences between controlled and uncontrolled patients, further support the suggestion that airway periostin is also a biomarker of severity of lung disease while it is less sensitive in monitoring of dynamic changes. Probably the signal that acts to increase the transcription, the production, and the release of periostin locally in airways from epithelial cells requires time and influences the significance of periostin concentration, as a static clustering marker.[17] Previous investigators reached similar conclusions and did not report any differences in EBC periostin with changes in asthma control, which is further confirmed in this study by the lack of correlation that we found between airway periostin levels and the Asthma Control Test.[9-16]

In contrast with Górska et al,[16] we found differences in periostin levels between patients with atopic and nonatopic asthma, which means that this marker particularly clusters the T2 endotype with the phenotype of early onset atopic severe asthma. Proof of this hypothesis might be that good responders to biologic therapy with omalizumab are those that present high levels of periostin in serum as demonstrated by the markers analysis of the EXTRA study.[5]

For the first time we found higher periostin concentrations in women compared with men. Ivancsó et al[18] demonstrated that pregnancy itself increases circulating periostin levels, and this elevation is detectable in pregnant patients with asthma as well. We did not correlate periostin with sex hormones. This correlation could better explain the higher production of this protein in women, which merits further study.

Although the precise mechanisms are unknown, elevated serum periostin levels are less frequently observed in patients who are obese with asthma,[19] which is also reported in a recent epidemiologic study on serum periostin levels. It is thought that periostin is released from airway epithelial cells as a result of stimulation by IL-4 and IL-13, through their common receptor IL-4Rα. Increased airway concentrations may therefore be particularly useful in predicting responses to anti-IL-13 or anti-IL-4Rα therapies.

We also explored the differences in patients who are and are not obese with asthma, but we were not able to find significant evidence.

In this study we analyzed periostin in the airways of healthy subjects and found, accordingly with previous results, low concentrations compared with patients with asthma,[16] which demonstrates the value of periostin as a sensitive and specific marker of asthma.

Values of periostin in airways were lower compared with values reported in blood by Scichilone et al,[8] but this was expected because of the dilution of the sample that comes from the target organ. In support of this explanation comes the strong expression of periostin at the RNA level in epithelial cells and not detestability of the protein expression in these cells previously reported.[20] The discrepancy between RNA and protein expression has led to the postulation that periostin protein is rapidly secreted by airway epithelial cells into the subepithelial layer.[21] The hypotheses is that periostin is abundant in the basal medium but could not be detected in the apical washes.[21] Therefore, its concentrations in airways are low when compared with blood; however, they are significantly higher than the detection limit of the ELISA kit used.[22]

In consideration of the high sensitivity of periostin in airways to detect patients with severe asthma compared

with patients with mild to moderate asthma, and particularly the T2 endotype compared with non-T2 endotype, we suggest that periostin in the EBC and in sputum may serve as a potential clinical marker for severe eosinophilic T2 asthma being more organ-targeted that serum periostin.[16]

Furthermore, EBC is a collection method from airways that is rapid, that is completely noninvasive, that can be used in patients with severe asthma, and that is safe. These characteristics may be important when the patients to be analyzed are those with severe and uncontrolled asthma that often is very difficult to monitor with other functional and biologic techniques. Our findings show that periostin concentrations in EBC reach values that may be detected by a commercially available ELISA kit. The correlation that we found between the periostin levels in EBC and IS supernatant indicates that we can use both samples, preferring the EBC in those patients with more severe uncontrolled asthma unable to perform IS, limiting the IS to patients with less severe asthma. These results may provide new opportunities for research, particularly in severe asthma, in which more invasive procedures (although considered relatively safe) carry the potential risk of airway obstruction and disease exacerbation.

A limitation of this study is the lack of correlation with the periostin concentration in paired serum samples. However, in this study we focused on airways, but we are planning to evaluate this aspect in a future study. Another limitation was we did not have samples collected at the same time of the day. Subjects were enrolled consecutively and during a routine visit and therefore within a range of 0 to 8 h during the day. In consideration of the recent finding of day-time variation of serum periostin in adults with asthma, with higher levels in the morning, we think it could be important to standardize collection at the same time of day in future studies to confirm this variability in patients with severe asthma.[7]

In conclusion, we confirmed that it is possible to study periostin in EBC and IS supernatant. For the first time, we analyzed periostin in airways of patients with severe asthma, dividing them into T2 and non-T2 endotypes, and found that airways periostin may be the expression of T2 severe asthma. Airways periostin might be a useful biomarker to apply stratified medicine for severe asthma and to yield better outcomes in asthma management. There is a need in searching for different panels composed by biomarkers like airways periostin to monitor treatment response in clinical practice.

Acknowledgments

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