ORIGINAL ARTICLE

Comparative study of periostin expression in different respiratory samples in patients with asthma and chronic obstructive pulmonary disease

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KEY WORDS

airways inflammation, asthma, chronic obstructive pulmonary disease, eosinophils, periostin

ABSTRACT

INTRODUCTION Periostin is considered to be a marker of eosinophilic inflammation in patients with asthma. However, there are no literature data on periostin in patients with chronic obstructive pulmonary disease (COPD).

OBJECTIVES The aim of the study was to evaluate periostin expression and to compare its concentrations in various materials in patients with mild-to-moderate asthma and COPD, as well as to evaluate the potential association between periostin and clinical features of both diseases.

PATIENTS AND METHODS Using an enzyme-linked immunosorbent assay, we measured periostin concentrations in serum, induced sputum (IS), exhaled breath condensate (EBC), and bronchoalveolar lavage fluid (BALF) as well as periostin expression in bronchial biopsy samples in 24 patients with asthma, 36 patients with COPD, and 12 controls. Correlations between periostin levels in different materials were also analyzed and periostin concentrations were compared between patients with asthma and those with COPD.

RESULTS Periostin levels were detectable in serum, IS, EBC, and BALF from patients with asthma, COPD, and controls. EBC periostin levels correlated with tissue periostin expression and were significantly higher in asthma than in COPD ($P = 0.04$). Periostin expression in bronchial mucosa was higher in asthma than in COPD ($P < 0.001$), as well as in asthma and COPD patients compared with controls ($P < 0.001$). No significant correlations between tissue periostin expression and BALF, IS, or serum periostin levels were found. There were no differences in serum, IS, BALF, or EBC periostin concentrations between patients with different phenotypes of both diseases.

CONCLUSIONS Periostin may be detected not only in serum, IS, and airway tissue samples, but also in EBC and BALF. EBC periostin levels and tissue periostin expression are higher in patients with asthma than in those with COPD. EBC periostin levels may serve as a potential surrogate marker for tissue periostin expression.

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INTRODUCTION Asthma is a chronic inflammatory disease with a complex immunological background. Eosinophils are the main cells involved in asthma-related airway inflammation; however, there is also a significant contribution of neutrophils, lymphocytes, and mast cells. As the type of inflammation has been shown to affect treatment response, determination of asthma inflammatory phenotypes based on the predominant inflammatory cells has been strongly advocated. In recent years, further attempts have been made to precisely identify these phenotypes, and this has initiated a search for new reliable biomarkers. Periostin has been found to be a marker for Th2-associated inflammation in patients with asthma.[1] This type of inflammation is associated with an increased eosinophil count not only in peripheral blood, but also in bronchoalveolar lavage fluid (BALF).[2] Therefore, periostin may be used as a potential predictor of airway eosinophilia.[3]

Periostin is an interleukin (IL)-4/IL-13-induced secreted extracellular protein with structural homology to adhesion molecule fasciclin I. It was originally isolated from an osteoblast cell line.[4] Studies have indicated that periostin is one of the most highly expressed genes in airway epithelial cells and lung fibroblasts in asthmatic airways.[5,6] Periostin enhances profibrotic tumor growth factor-β signaling in subepithelial fibrosis associated with remodeling in asthma.[7] Earlier studies demonstrated higher periostin concentrations in the serum of patients with eosinophilic asthma compared with those with noneosinophilic asthma[3,8] and its elevated expression in sputum cells in asthmatic patients compared with healthy subjects.[9] These materials are easily accessible and obtained by noninvasive methods; however, they may not precisely reflect the inflammatory status in the airways and lung parenchyma. In a recent study, Hastie et al[10] have shown that serum markers do not accurately predict the cellular content of sputum in asthmatic patients. Airway epithelial cells have a high expression of periostin but periostin is secreted mainly in the basal direction,[7] and it is not clear whether the secretion to the airway lumen is uniform at all airway levels. Therefore, there is a need for studies that would systematically evaluate periostin concentrations in different respiratory samples, determine their mutual relations, and more precisely determine the role of periostin in airway inflammation.

Although airway eosinophilia and remodeling are the hallmarks of asthma,[11] some patients present with noneosinophilic inflammation.[12,13] Neutrophilic asthma is a distinct asthma phenotype with poor steroid response and evidence of systemic inflammation, both of which are common features of chronic obstructive pulmonary disease (COPD).[14] Neutrophils are considered the key inflammatory cells in COPD, but as much as 20% to 40% of patients with COPD may have elevated sputum eosinophil counts and eosinophils in airway biopsy samples.[15,16] Given the elevated periostin concentrations not only in eosinophilic but also in neutrophilic asthma and the documented contribution of eosinophils in COPD, we may assume that periostin is also involved in the pathogenesis of COPD.[13] However, there are no literature data on periostin expression in patients with COPD. Therefore, we aimed to evaluate periostin expression and compare its levels in various materials from patients with mild-to--moderate asthma and COPD, as well as to assess the relationship between periostin expression and clinical features of asthma and COPD.

PATIENTS AND METHODS

This prospective cross--sectional study was performed between 2012 and 2014 and included 24 patients with mild-to-moderate asthma, 36 patients with mild-to-moderate COPD (Global Initiative for Chronic Obstructive Lung Disease [GOLD] classification, stages I–II), and 12 control subjects.

Patients were recruited from an outpatient clinic and included consecutive patients with asthma and COPD who were asked to participate in the study during a routine control visit and had signed an informed written consent form. Only patients who had not been treated with inhaled or oral steroids within 6 weeks before enrollment and who had not experienced disease exacerbation or respiratory infection 6 weeks before the study onset were included.

The study project was approved by the institutional review board and registered at ClinicalTrial.gov (NCT02069054).

Definitions The assignment to a specific study group (asthma or COPD) was based on past medical history, clinical signs and symptoms, and the results of the following examinations: spirometry with a bronchial obstruction reversibility test performed according to the European Respiratory Society (ERS) guidelines,[17] methacholine bronchial challenge,[18] blood laboratory tests (absolute eosinophil count and percentage; the cut-off level for serum eosinophilia was determined at 0.3×10^9/l), and allergy skin prick tests. The severity of airflow limitation was evaluated in accordance with the ERS guidelines.[19] Atopy was defined as the presence of at least 1 positive result of the skin prick test to common aeroallergens, with a diameter of 3 mm or greater than the positive control.[20]

The diagnosis of asthma and its severity were established in accordance with the Global Initiative for Asthma guidelines,[21] and the diagnosis of COPD—in accordance with the GOLD guidelines.[22] The number of exacerbations in the past year was recorded, and disease control was assessed by the Asthma Control Test (ACT) in asthmatics and by the COPD Assessment Test in COPD patients, respectively.

All control subjects had a negative history of atopy or obstructive lung disease, had normal spirometry results, and PC_{20} methacholine levels exceeding 16 mg/ml.

The exclusion criteria for all subjects were as follows: use of inhaled or oral steroids (or both) 6 weeks before the study, respiratory tract infection, exacerbation of asthma or COPD within 6 weeks before the study.

Sputum induction and processing Sputum induction was performed with sterile hypertonic saline (NaCl) at increasing concentrations (3%, 4%, and 5% solutions) via an ultrasonic nebulizer (ULTRA-NEB™2000, DeVilbiss Healthcare, United States) as described previously.[23] Plugs were isolated from saliva and were processed with 0.1% solution of dithiothreitol (DTT, Sigma Aldrich Co. St. Louis, Missouri, United States). Induced sputum (IS) samples were processed and examined for nonsquamous cell counts by means of cytospin and visual count, as previously described.[24-27] The differential cell count was determined in May–Grünwald–Giemsa-stained

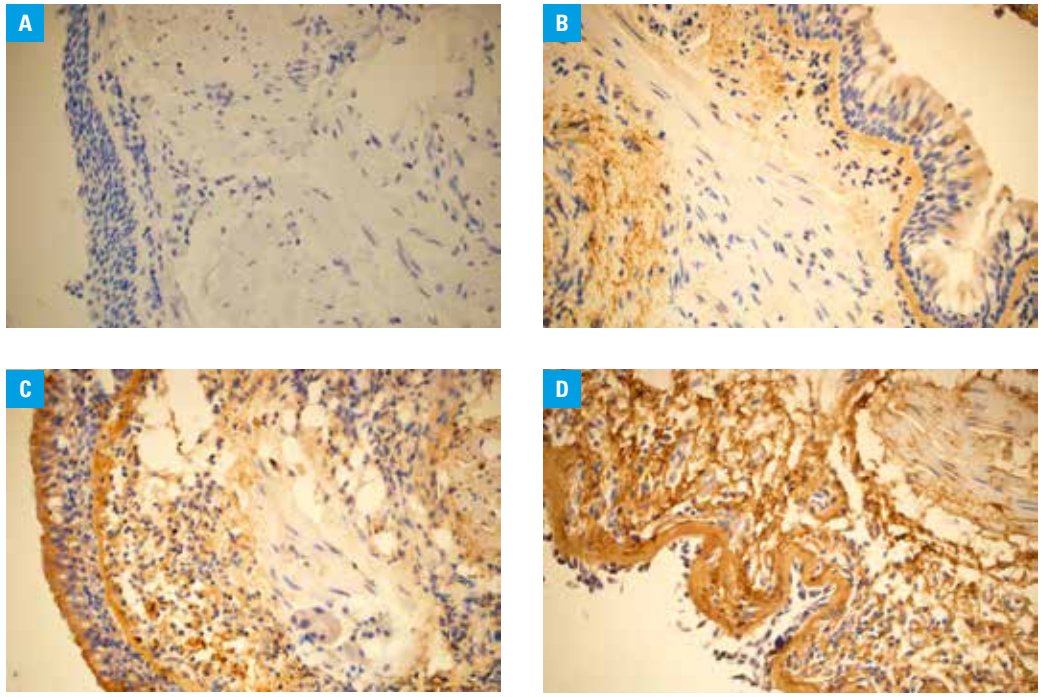


FIGURE 1 Periostin expression in bronchial mucosa samples (brown stripe below the epithelial layer): examples of rating based on the intensity of immunostaining (**A** – no staining; **B** – 1+, weak; **C** – 2+, intermediate; and **D** — 3+, strong)

smears, based on the morphology of 300 influx cells from various fields. Subjects with sputum eosinophil count of 3% or higher were classified as having sputum eosinophilia.[10,28] Supernatants were collected and stored at –70°C for periostin measurements.

Exhaled breath condensate collection Exhaled breath condensate (EBC) was collected and processed according to the American Thoracic Society / ERS recommendations,[29] using the TURBO-DECCS 09 system (Medivac, Parma, Italy). In all subjects, EBC was collected between 10:00 and 11:00 AM and obtained during tidal breathing. At the end of collection, 1.5 to 3.5 ml aliquots of EBC were transferred to Eppendorf tubes and immediately frozen at –70°C.

Fiberoptic bronchoscopy, bronchoalveolar lavage, and bronchial biopsies Flexible bronchoscopy was performed under local anesthesia (2% lidocaine) after premedication with inhaled salbutamol (400 μg), atropine (0.5 mg intramuscularly), and midazolam (7.5 mg orally). Bronchoalveolar lavage (BAL) of the middle lobe was performed by administration of 4 × 50 ml of sterile 0.9% NaCl warmed to 37°C. The subsequent steps of BALF processing were completed according to a previously published protocol.[30,31] Total cell count and cellular composition were assessed in the sediment after BALF centrifugation. Subjects with BALF eosinophil count of 2% or higher were classified as having BALF eosinophilia.[32]

After BAL, 2 to 4 bronchial forceps biopsies were taken from the segmental and subsegmental bronchi of the right lower lobe. Freshly obtained biopsy specimens from the bronchial mucosa were fixed in 10% formalin, routinely processed, embedded in paraffin wax, and stained with hematoxylin and eosin to identify and quantify eosinophils. Immunohistochemical staining with polyclonal antiperiostin antibody (Ab14041, dilution 1:1000, Abcam, United Kingdom) was applied to 5-μm thick paraffin-embedded sections according to the manufacturer's instructions. The EnVision Detection System (Dako Denmark A/S, Glostrup, Denmark) was used for detection. For validation of periostin staining, human breast cancer tissue was evaluated. Negative (isotype) controls were performed using a ready-to-use FLEX Negative Control Mouse (cocktail of mouse IgG1, IgG2a, IgG2b, IgG3 and IgM; code No IR750; Dako Denmark A/S).

All stained sections were photographed at ×200 magnification and analyzed with the acquisition software of the CellSens package (Olympus, Japan). To quantify eosinophils in the bronchial mucosa, all slides were assessed to select the section with the most prominent inflammatory infiltrates, and this section was used to evaluate the eosinophil count. Eosinophils were counted in an area of 0.948 mm^2. The eosinophilic cut-off point for the bronchial biopsy specimens of 2 cells/mm^2 was established.[9]

Periostin analysis Periostin levels were measured in thawed serum (×10 dilution) and undiluted EBC, sputum, and BALF supernatants (Periostin/OSF-2 human ELISA kit, Phoenix Pharmaceuticals, United States). The range of the standard curve was from 0.027 to 20 ng/ml.

Periostin expression in the bronchial biopsy specimens was evaluated with semiquantitative method (FIGURE 1) based on the intensity of immunostaining on the extracellular space and extracellular matrix.[33]

Statistical analysis **Sample size estimation** Estimation of the sample size was based on our preliminary data from the first 10 asthmatic and 19

TABLE 1 Demographic and basic clinical data of patients with asthma, chronic obstructive pulmonary disease, and controls

Parameter		Asthma (n = 24)	COPD (n = 36)	Control (n = 12)	*P* value
sex, male/female, n		11/13	21/15	6/6	0.34
age, y, median (IQR)		51 (31–60.5)	66.5 (60–72)	54.5 (36–63.5)	<0.001[a] 1.0[b] 0.005[c]
BMI, kg/m^2, median (IQR)		27.1 (25.5–31.2)	26.3 (23.2–29.8)	27.7 (24.5–30.8)	0.28
never-smokers/ ex-smokers/ current smokers, n (%)		21 (87.5)/3 (12.5)/0	0/20 (55.5)/16 (44.5)	7 (58)/3 (25)/2 (17)	<0.001
smoking history, pack-years, median (IQR)		0 (0.0–0.0)	45 (33.5–60)	0 (0–20)	<0.001[a] 1.0[b] <0.001[c]
duration of symptoms, mo, median (IQR)		97 (33–120)	60 (24–120)	–	0.87
positive skin prick-tests, n (%)		15 (62.5)	7 (19)	2 (17)	<0.001
prebronchodilatory FEV_1, median (IQR)	l	2.61 (1.91–3.91)	1.59 (1.23–1.95)	2.86 (2.06–3.84)	<0.001[a] 0.4[b] <0.001[c]
	% pred.	88.5 (75.5–97)	65.5 (54–74.35	99 (90–107)	
postbronchodilatory FEV_1, median (IQR)	l	2.67 (2.1–4.3)	1.78 (1.31–2.2)	–	<0.001
	% pred.	98.5 (85–103)	70 (63.5–80)	–	
fixed obstruction[d], n (%)		8 (33)	36 (100)	–	<0.001

For comparisons in which the Fisher exact test was used, as well as for those with insignificant results of the Kruskall–Wallis test, only 1 *P* value is shown; for comparisons with significance in the Kruskal–Wallis test, *P* values are shown for each compared pair:

a asthmatics vs COPD; **b** asthmatics vs controls; **c** COPD vs controls.

d defined as postbronchodilator FEV_1/FVC <5th percentile

Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 second, FVC, forced expiratory volume; IQR, interquartile range

COPD patients enrolled in the first 10 months of the study. We used the results of the measurement of EBC periostin levels in asthma and COPD (1.30 ±0.51 ng/ml and 0.32 ±0.80 ng/ml, respectively). Although EBC periostin concentrations in patients with asthma were more than 4-fold higher than those in patients with COPD, we used a 2-fold difference between the groups and the higher value of standard deviation, found in patients with COPD (0.80), for the calculation of the sample size. To detect the difference with a power of 80% and a significance level of 5%, the sample size was estimated as 54 patients, including 18 patients with asthma and 36 patients with COPD.

Data presentation and statistical analysis Data were expressed as medians and interquartile ranges (IQRs) (25th to 75th percentiles) or numbers and percentages.

The statistical analysis was performed using Statistica 10.0 (StatSoft Inc., Tulsa, Oklahoma, United States) and MedCalc Statistical Software version 13.2.2 (MedCalc Software bvba, Ostend, Belgium). Quantitive data distribution was assessed using the Shapiro–Wilk test. The differences between continuous variables in the 2 groups were tested using the nonparametric Mann–Whitney test. The Kruskal–Wallis test with the subsequent use of the post-hoc Dunn test (for multiple comparisons) was applied when continuous variables in more than 2 groups were compared. Categorical variables were compared using the Fisher exact test. The strength and direction of the relationship between 2 variables was measured with the Spearman rank correlation coefficient. Statistical significance was accepted at a *P* value of less than 0.05.

RESULTS **Characteristics of patients** The basic clinical characteristics of patients with asthma, COPD, and controls are presented in TABLE 1.

Eosinophils The absolute eosinophil count in IS and bronchial mucosa was significantly higher in asthmatics than in patients with COPD and controls (TABLE 2). Significant correlations between blood and sputum eosinophils, blood and BALF eosinophils, as well as sputum and BALF eosinophils were found in asthmatic patients (FIGURE 2). In contrast, none of the above correlations were significant in COPD patients.

In asthmatic patients, tissue eosinophils did not correlate with the absolute eosinophil count in peripheral blood (r = 0.09; P = 0.7), IS (r = 0.34; P = 0.1), and BALF (r = –0.03; P = 0.9).

Periostin Serum periostin concentrations were 75- to 600-fold higher than those found in IS, BALF, and EBC (P <0.001 for all comparisons between serum periostin levels vs periostin in IS, BALF, and EBC); however, there was no difference in serum periostin levels between all the 3 study groups. Asthmatic patients showed significantly higher sputum periostin levels than COPD patients

TABLE 2 Peripheral blood and airway eosinophil count as well as periostin concentrations in the study groups

Variable	Asthma (n = 24)	COPD (n = 36)	Controls (n = 12)	*P* value
eosinophil count				
blood eosinophils, $\times 10^9$/l	0.22 (0.16–0.37)	0.14 (0.09–0.22)	0.16 (0.07–0.24)	0.056
sputum eosinophils (% of nonsquamous cells)	10 (2–28)	1.5 (0–5)	1 (1–2)	<0.001[a] 0.005[b] 1.0[c]
BALF eosinophils (% of nonepithelial cells)	1.5 (0.0–3.5)	0 (0–1)	0.5 (0–1)	0.06
eosinophil count in biopsy specimen, n/mm^2	9.5 (5.3–15.8)	2.1 (0.0–2.1)	0.0 (0.0–3.2)	<0.001[a] <0.001[b] 1.0[c]
periostin concentrations				
serum periostin, ng/ml	122.1 (99.2–139.2)	124.2 (102.3–154.7)	135.2 (128.1–146.3)	0.12
IS periostin, ng/ml	0.7 (0.2–6.5)	0.3 (0.1–1.2)	1.8 (0.4–5.0)	0.12[a] 1.0[b] 0.04[c]
EBC periostin, ng/ml	1.5((0.8–1.6)	0.2((0.1–0.3)	0.3 (0.3–0.8)	<0.001[a] 0.2[b] 0.046[c]
BALF periostin, ng/ml	0.3 (0.16–0.5)	0.1 (0.02–0.6)	0.08 (0.04–0.3)	0.64

The results are presented as median (IQR).

For comparisons with no significance in the Kruskal–Wallis test, only 1 *P* value is shown; for comparisons with significance, *P* values are shown for each compared pair:

a asthmatics vs COPD; **b** asthmatics vs controls; **c** COPD vs controls.

Abbreviations: BALF, bronchoalveolar lavage fluid; EBC, exhaled breath condensate; IS, induced sputum; others, see TABLE 1

(*P* = 0.04). EBC periostin levels were also higher in asthmatics, and the difference was significant when compared both with COPD patients and with controls (TABLE 2). No correlations between IS and EBC periostin levels in any of the study groups were found.

The lowest periostin levels were found in BALF. BALF periostin levels correlated with sputum periostin levels in asthmatic patients; however, no such correlation was observed for EBC (FIGURE 3). In patients with COPD, significant correlations were found between periostin levels in BALF, serum, and EBC.

The periostin expression in bronchial mucosa samples differed significantly between the study groups. Almost equally high periostin expression was found in all patients with asthma: in 18 patients (78%), it was expressed as 3+ and in 5 patients (22%), as 2+ (FIGURE 4). Periostin expression in patients with COPD was significantly lower (*P* <0.001). The mucosal expression of periostin was rated as 2+ in 22 patients (61%) and as 1+ in 13 patients (36%) with COPD. Only 1 patient with COPD showed the periostin expression of 3+. A significantly lower periostin expression was demonstrated in bronchial mucosa samples obtained from controls compared with those obtained from asthmatic and COPD patients (*P* <0.001).

Tissue periostin expression did not correlate with BALF, IS, or serum periostin levels either in the whole study group or in any of the groups when analyzed separately. However, it correlated with EBC periostin concentrations (*r* = 0.4, *P* = 0.003) in the whole group. The correlation between the periostin expression and EBC periostin levels was more pronounced in patients with asthma and COPD (*r* = 0.5, *P* <0.001), but was not found for either asthmatic or COPD patients when analyzed separately.

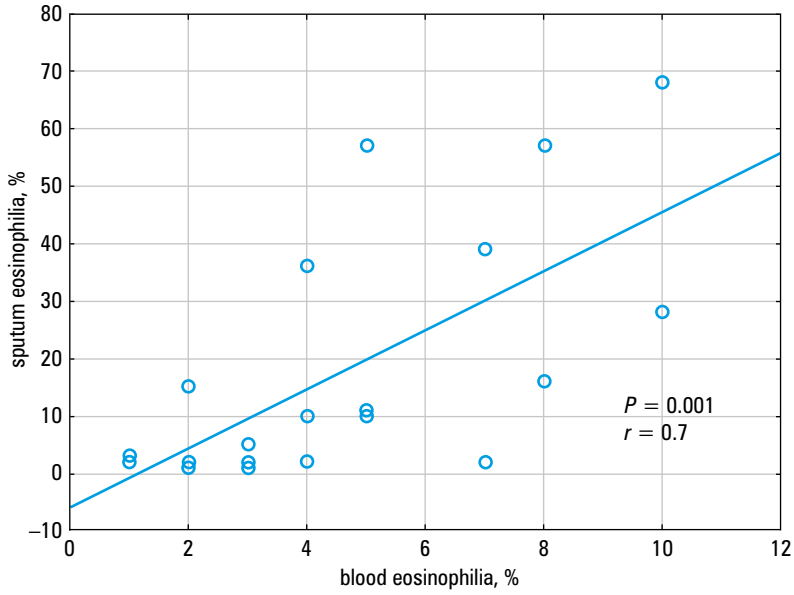
In asthmatic patients, no correlations between tissue periostin expression and blood, IS, or BALF eosinophil count were found.

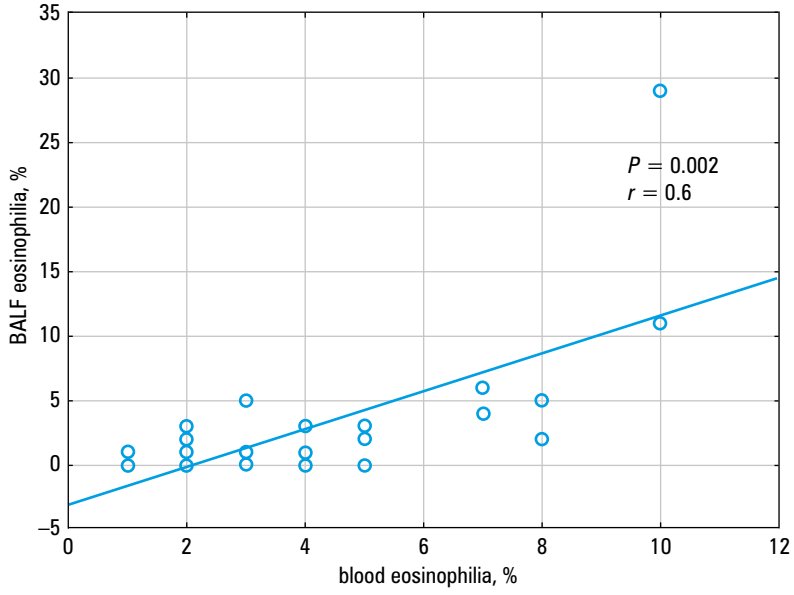
In patients with COPD, the absolute neutrophil count in BALF was significantly higher in patients with low mucosal periostin expression as compared with those with high mucosal periostin expression: 0.83 × 10^6/ml (IQR, 0.53–1.14) vs 0.39 × 10^6/l (IQR, 0.25–0.56), respectively (*P* = 0.03). Moreover, a negative correlation between tissue periostin expression and BALF neutrophil count was found (*r* = –0.4; *P* = 0.02). Such a correlation was not observed in asthmatic patients.

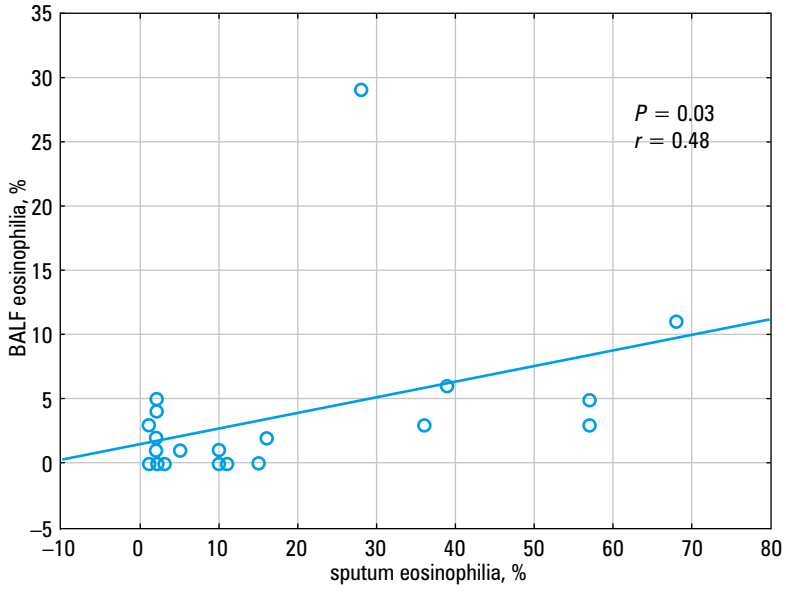
Correlations between periostin expression and concentrations and eosinophilia To assess the potential associations between periostin and eosinophilia, patients were divided into 2 subgroups, eosinophilic and noneosinophilic, with respective discriminating criteria for the analyzed materials as described above. We did not find any significant differences in serum periostin concentrations

FIGURE 2
Correlations between the eosinophil count in peripheral blood, induced sputum, and bronchoalveolar lavage fluid (BALF) in patients with asthma (**A**) and chronic obstructive pulmonary disease (**B**) (continued on the next page)

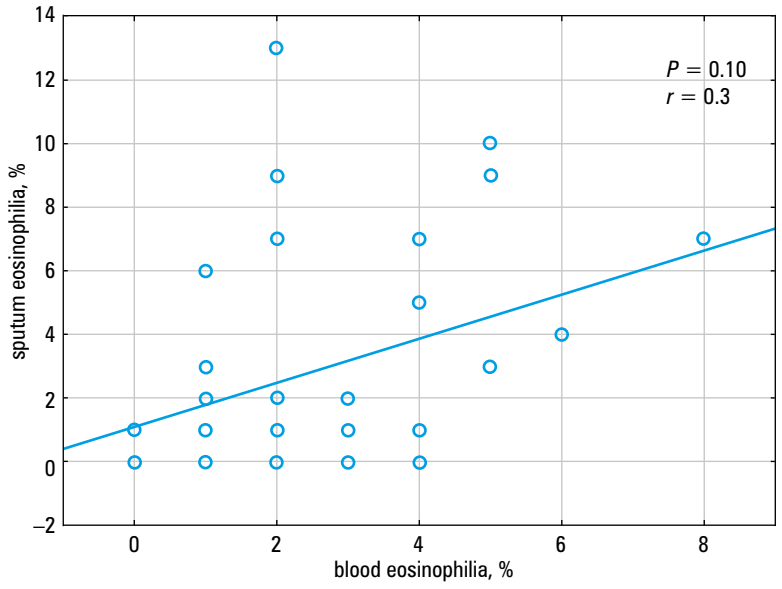
A

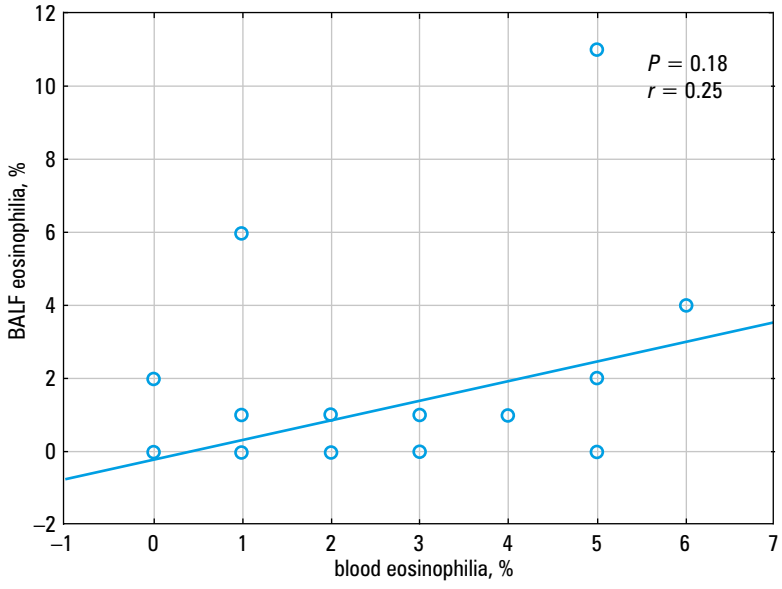






B


P = 0.10
r = 0.3
sputum eosinophilia, %
blood eosinophilia, %


P = 0.18
r = 0.25
BALF eosinophilia, %
blood eosinophilia, %

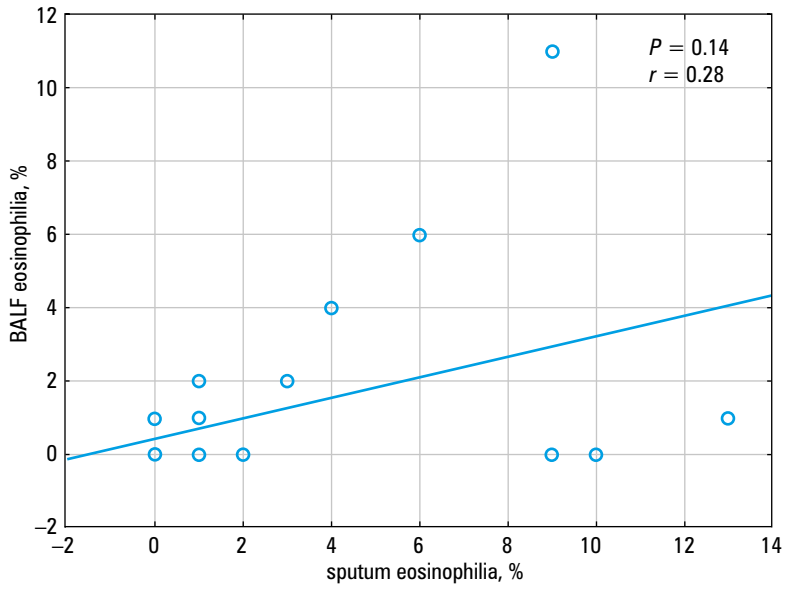
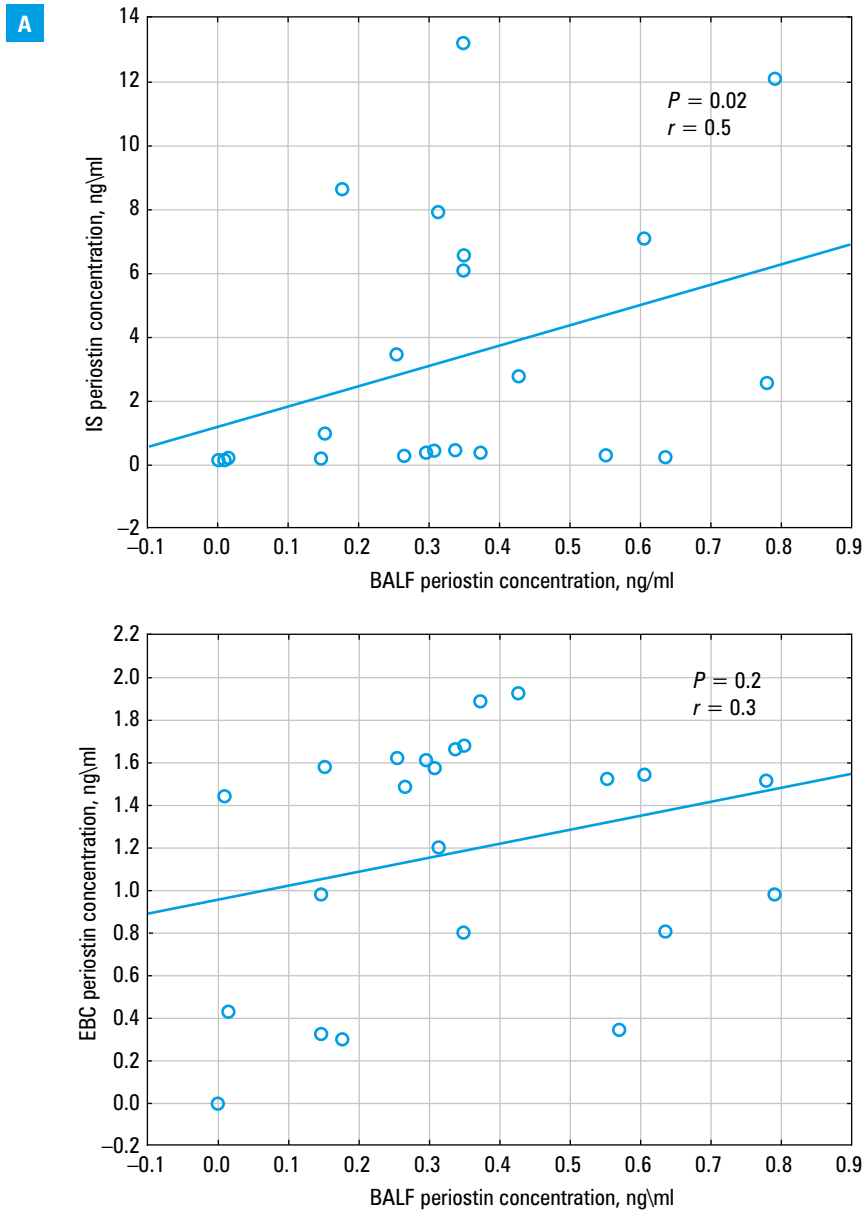

P = 0.14
r = 0.28
BALF eosinophilia, %
sputum eosinophilia, %

FIGURE 3 Correlations between periostin levels in bronchoalveolar lavage fluid (BALF) and those in induced sputum (IS), exhaled breath condensate (EBC), and serum in patients with asthma (**A**) and chronic obstructive pulmonary disease (**B**) (continued on the next page)

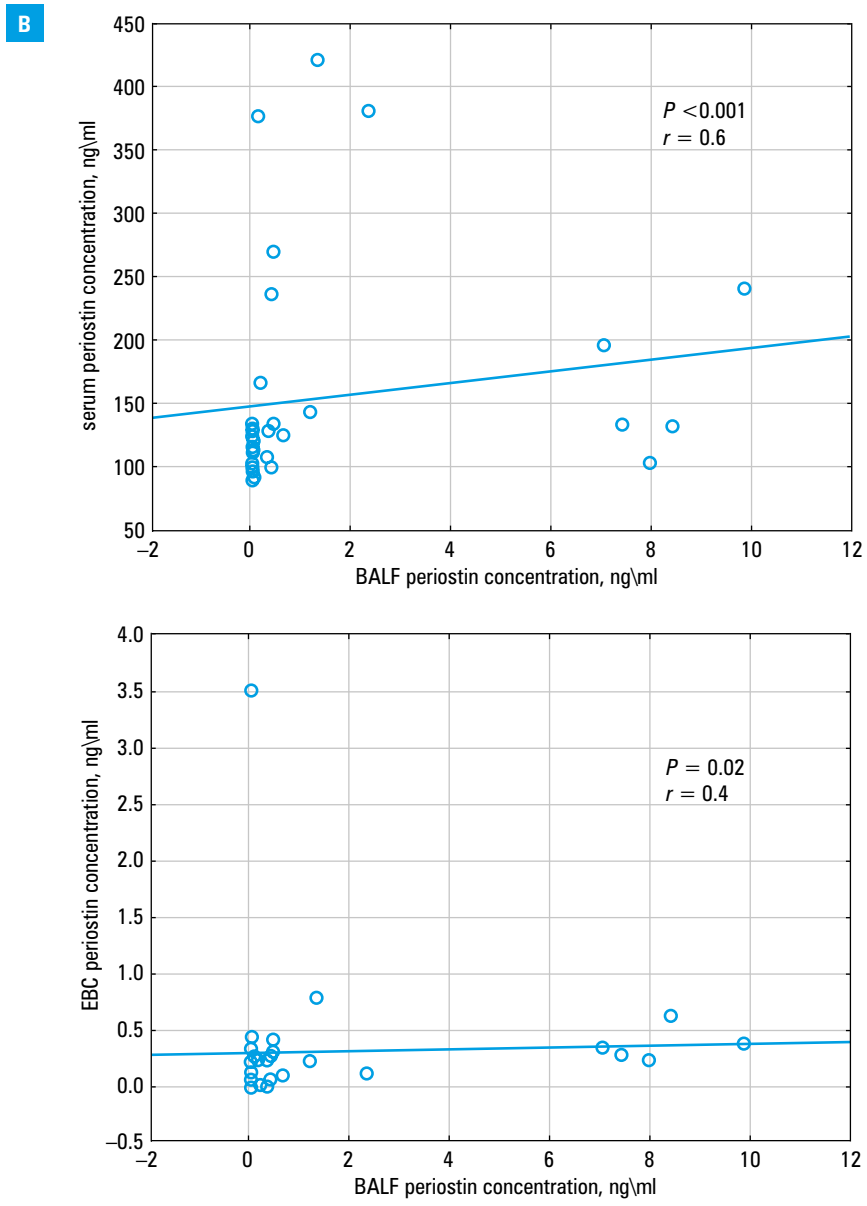


between the subgroups (eosinophilic subgroup, 131.8 ng/ml [IQR, 113.3–189.5 ng/ml]; noneosinophilic subgroup, 124.7 ng/ml [IQR, 103.1–141.1 ng/ml]; $P = 0.3$). Similarly, IS periostin levels did not differ between patients with and without sputum eosinophilia. In addition, we did not find any differences in BALF periostin levels between patients with and without BALF eosinophilia. This was also observed for the group of asthmatic patients alone.

The only difference between patients with and without tissue eosinophilia was found for periostin levels in EBC. This difference was significant for the group as a whole, but not for asthmatic patients alone (TABLE 3).

We also performed separate analyses for composite scores comprising of at least 2 or 3 investigated materials with eosinophilia; however, no differences were found in periostin concentrations or expression between the groups with and without the scores. The relationship between tissue periostin expression and eosinophil count in bronchial biopsy samples in patients with asthma and COPD is presented in FIGURE 5. The degree of the periostin expression did not correlate with tissue eosinophil count. In general, higher tissue eosinophilia was observed in asthmatic patients (compared with COPD patients) regardless of tissue periostin expression. However, there were also patients with COPD and low eosinophil count who showed moderate (2+) or high (3+) tissue periostin expression.

Periostin and disease phenotypes Although the number of patients with different asthma and COPD phenotypes was small, we performed a separate analysis of periostin levels in these subgroups. We did not find any differences in periostin levels between the studied samples in asthmatic patients with 4 different inflammatory phenotypes defined by cytological sputum characteristics: eosinophilic (≥3% eosinophils, n = 7), neutrophilic (≥40% neutrophils, n = 5), mixed granulocytic (≥3% eosinophils and >40% neutrophils, n = 5), and paucigranulocytic (<3% eosinophils and <40% neutrophils, n = 3). The



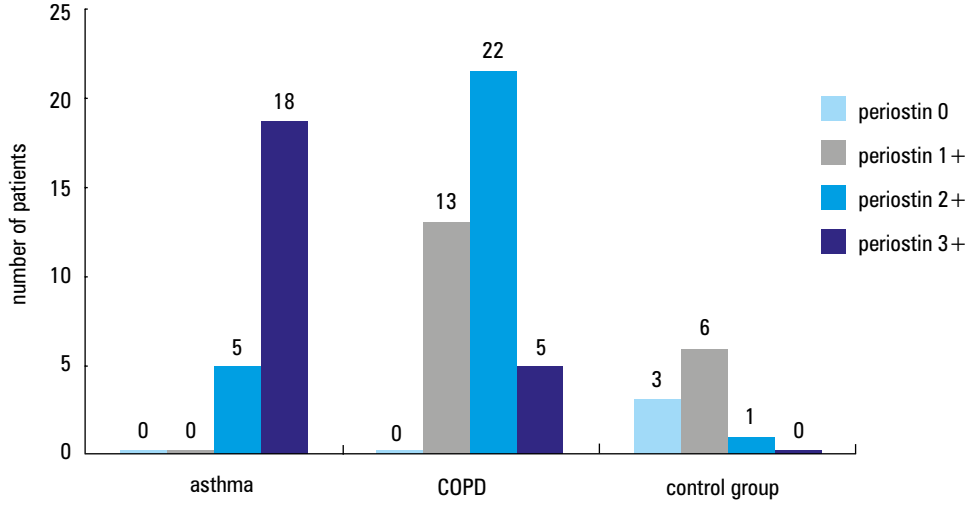


FIGURE 4 Distribution of tissue periostin expression in bronchial mucosa biopsy samples in patients with asthma (n = 23), chronic obstructive pulmonary disease (COPD) (n = 36), and controls (n = 10)

same was observed for other phenotypes: atopic vs nonatopic, persistent (postbronchodilatory forced expiratory volume in 1 second [FEV_1] to forced expiratory volume ratio, <5th percentile) vs nonpersistent (variable) airflow limitation, and obesity-related asthma (body mass index ≥30 kg/m^2). Lower BALF periostin levels were found in individuals with late-onset asthma (ie, aged ≥40 years, n = 12) when compared with those with early-onset asthma (n = 12): 0.21 ng/ml (IQR,

TABLE 3 Periostin levels in exhaled breath condensate in relation to the presence or absence of differently defined eosinophilia

Definition of eosinophilia	EBC periostin level (ng/ml) in patients with COPD, asthma, and controls			EBC periostin level (ng/ml) in asthmatic patients		
	noneosinophilic subjects	eosinophilic subjects	*P* value	noneosinophilic patients	eosinophilic patients	*P* value
tissue samples (cut-off, 2/mm^2)	0.28 (0.21–0.34)	0.43 (0.22–1.54)	<0.001	0.33 (0.30–1.48)	1.52 (0.98–1.62)	0.08
blood (cut-off, 300/ml)	0.3 (0.2–0.8)	0.8 (0.2–1.5)	0.37	1.52 (0.4–1.6)	1.21 (0.8–1.56)	0.60
IS (cut-off, 3%)	0.47 (0.19–1.44)	0.31 (0.22–0.77)	0.31	1.56 (1.25–1.6)	1.32 (0.8–1.56)	0.32
BALF (cut-off, 2%)	0.31 (0.23–0.98)	0.64 (0.28–1.49)	0.30	1.33 (0.98–1.68)	1.21 (0.62–1.53)	0.11

Data are presented as median (IQR).

Abbreviations: see **TABLES 1** and **2**

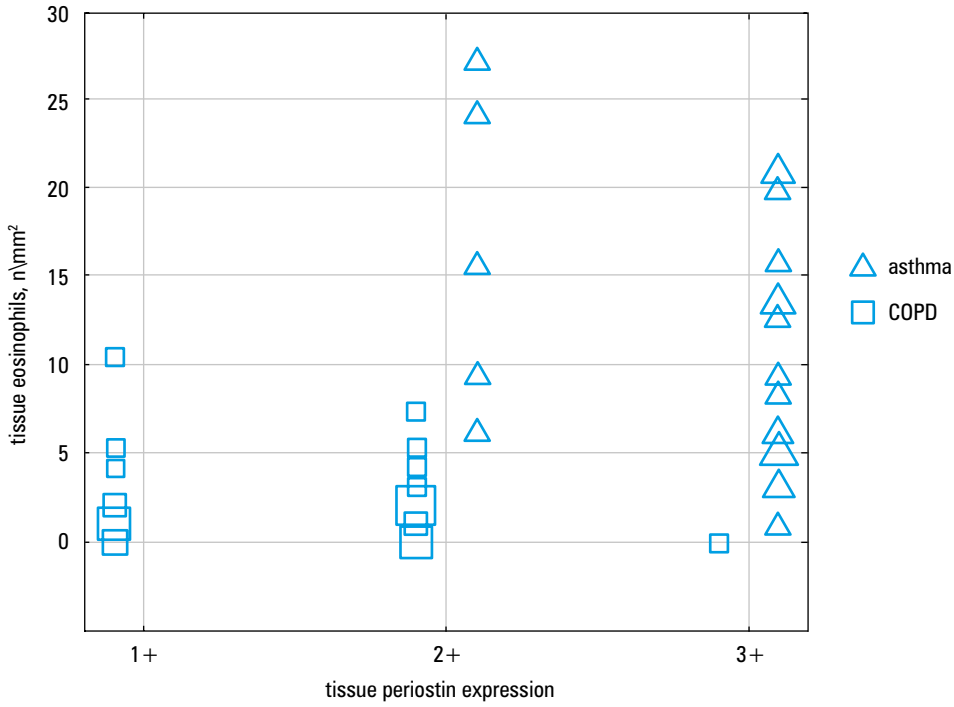


FIGURE 5 Correlation between periostin expression in bronchial mucosa samples and tissue eosinophilia in patients with asthma and chronic obstructive pulmonary disease; the size of the square/triangle corresponds with the number of patients.

0.15–0.34 ng/ml) vs 0.36 ng/ml (IQR, 0.31–0.62 ng/ml), respectively (P = 0.02). Higher BALF periostin levels were found in patients with worse asthma control (ACT score <20 points) when compared with those with well-controlled asthma (ACT score ≥20 points): 0.35 ng/ml (IQR, 0.31–0.63 ng/ml) vs 0.25 ng/ml (IQR, 0.14–0.33 ng/ml) (P = 0.01). Periostin concentrations and tissue periostin expression did not correlate with lung function and PC_{20}. We also failed to confirm the effect of such comorbidities as gastroesophageal reflux disease or chronic sinusitis on periostin levels in the investigated materials.

No differences in tissue periostin expression were noted between smokers and ex-smokers (P = 0.8); however, the periostin expression correlated with indices of hyperinflation, namely, residual volume / total lung capacity (r = 0.4; P = 0.048). No such correlation was observed in asthmatic patients.

DISCUSSION The present study has shown that periostin levels are detectable in serum, IS, EBC, and BALF obtained not only from patients with asthma but also from those with COPD, with significant differences between the 2 groups. However, we did not find any correlations in periostin concentrations between the investigated materials or any significant correlations between periostin levels and tissue expression.

Our report has provided some novel findings in comparison with previous studies on periostin. First, to our knowledge, this is the only study to date that enables a direct comparison of periostin concentrations in various respiratory samples. Second, we have not identified any previous studies that would measure periostin levels in EBC. Third, as we were not able to find any other data on periostin in patients with COPD, we believe this is the first study addressing this issue. Finally, our study focused on patients with mild-to-moderate disease who did not use steroids for at least 6 weeks, while the majority of previous studies had been performed in patients with severe/refractory asthma treated with steroids.

A number of studies have shown that periostin levels in serum and IS, as well as tissue periostin expression in the airways are elevated in severe refractory asthma with eosinophil predominance.[3,34] In asthmatics, the serum periostin level is associated with a more rapid decline in FEV_1 and bronchial hyperresponsiveness.[34,35] In our patients with mild-to-moderate asthma, no relationship between lung function or PC_{20} was found; however, BALF periostin levels were significantly higher in patients with an ACT score of less than 20 points. Furthermore, we did not find any correlations between periostin levels and asthma inflammatory phenotype. These findings are in accordance with the results of Wagener et al[36] who found that in mild-to-moderate asthma, the serum periostin level was a weak predictor of eosinophilic asthma. It must be emphasized that the nonsignificant associations between the disease phenotype and periostin level in various biological samples might have been related to the small number of patients in our study. Of note, in 2 asthmatic patients with high tissue eosinophilia (>22/mm^2) from our series, the periostin expression was not the highest and was rated as 2+. This may suggest that eosinophilic asthma is not the only inflammatory asthma phenotype associated with higher periostin expression.

To our knowledge, this is the first report on periostin in EBC. Despite a thorough literature search, we failed to find data on periostin levels in EBC either in asthma or other respiratory diseases. Reports on periostin come mainly from studies in asthmatic patients and concerned serum, IS, and tissue samples from the airways.[2,6,9,35] In our study, we demonstrated that measurable concentrations of periostin may be found in EBC from asthmatics, COPD, and controls, with the highest levels detected in patients with asthma, regardless of the inflammatory phenotype. These findings showed that periostin concentrations in EBC reach values that may be detected by commercially available enzyme-linked immunosorbent assays (ELISAs). It is believed that EBC reflects the molecular environment of the lower respiratory tract, whereas IS consists of cells and secretions derived mainly from the large airways.[37] Our study showed a correlation between EBC periostin levels and tissue periostin expression for the whole study group (asthma, COPD, and controls) as well as for patients with asthma and COPD analyzed together. We did not demonstrate such a correlation when COPD and asthma patients were analyzed separately, but this might have been caused by a relatively small number of patients in both groups. Our findings may provide new opportunities for research, particularly in asthma, in which sputum induction or invasive procedures (although considered relatively safe) carry the potential risk of airway obstruction and disease exacerbation.[38,39] Moreover, as our results demonstrated that EBC periostin levels are significantly higher in asthmatic patients than in those with COPD, it may serve as an additional easily accessible marker for differentiating asthma and COPD. Given the lack of data on EBC periostin levels, we could not compare our findings with those of other authors. In this context, the periostin level in different respiratory samples in patients with asthma–COPD overlap syndrome might be an interesting issue. However, the aim of our study was to compare periostin levels only in asthma and COPD, and the inclusion/exclusion criteria were clearly formulated to exclude patients with coexisting features of both diseases. Further studies are needed to elucidate this issue.

Reports on periostin levels in BALF are also very scarce. Some data come from studies involving patients with interstitial lung diseases (ILDs). Okamoto et al[40] analyzed the usefulness of serum and BALF periostin levels in differentiating ILD; however, the authors only reported that periostin was detectable in 5 of 11 patients with idiopathic pulmonary fibrosis and did not provide any numerical data. Recently, Nakamura et al[41] published an analysis of BALF periostin levels in 10 asthmatic patients. Initially periostin could be detected only in 3 subjects, but a 10-fold BALF protein concentration resulted in the detection of periostin in 8 patients. However, in 9 of 10 healthy controls periostin still could not be measured. Nevertheless, the authors demonstrated a correlation between serum and BALF periostin levels, which was also confirmed in our study. No data on BALF periostin levels in patients with COPD are available. In the present study, we found 3-fold lower BALF periostin levels in patients with COPD when compared with asthmatic patients; however, the difference was not significant, and the values did not differ significantly from those in controls.

Our study may be considered as a preliminary report on periostin levels in patients with COPD. The results indicate that periostin may be involved in airway inflammation in this disease. Interestingly, in this patient group tissue periostin expression was higher than in controls: in as many as 61% of patients with COPD the expression was 2+. This may be at least partially explained by the involvement of periostin in lung repair mechanisms and remodeling.[42] Furthermore, periostin expression tended to be lower in COPD patients with BALF neutrophilia. Periostin is known to enhance the migration and adhesion of eosinophils stimulated by IL-5, IL-3, and granulocyte-macrophage colony-stimulating factor in vitro[43]; therefore, we may speculate that eosinophil migration in COPD is limited because of the lack or lower levels of these stimulants or perhaps because of the presence of yet unknown inhibiting factors, and hence the low eosinophil counts and neutrophil predominance in the airways of COPD patients.

Smoking status did not influence the periostin expression or periostin levels in the investigated samples. This is in contrast to the hypothesis that smoking may affect periostin levels in

patients with obstructive lung diseases. Thomson et al[44] found lower serum periostin levels in asthmatic smokers when compared with never-smokers; however, these authors did not assess tissue periostin expression.

High tissue periostin expression did not correspond with its high levels in the investigated samples. This is in accordance with the findings of Sidhu et al[7] who demonstrated that the epithelial secretion of periostin has mainly basal not apical direction. Nakamura et al,[41] who investigated periostin levels in BALF from stable asthmatic patients and healthy subjects, even postulated that periostin secretion to the airway lumen may be considered negligible. Low periostin levels in IS in our patients may also be attributed to the use of DTT during sputum processing, as periostin is DTT--sensitive due to the presence of disulphide links in its structure. Periostin concentrations were the lowest in BALF, which may probably be explained by its high dilution. However, they were within the range of the standard curve of the applied ELISA kit, and this range (0.027–20 ng/ml) was comparable to that of the kits most frequently used by other authors.[45]

The lack of evident correlations between periostin levels and disease phenotypes in our study is somewhat disappointing. As already mentioned, this may be at least partially explained by the small number of patients with different disease features. It should be acknowledged that our study was underpowered in the context of evaluating the relationships between periostin levels and different phenotypes of asthma and COPD. The small number of participants, particularly those with different asthma and COPD phenotypes, might be considered the major limitation of our study. However, we would like to stress that since consecutive patients without any preselection were enrolled, our study presents real-life conditions. Participation in the study required bronchoscopy, to which patients were reluctant. Furthermore, the requirement of not using steroids for 6 weeks prior to the study onset limited the number of enrolled patients, particularly asthmatics.

In conclusion, periostin may be detected not only in serum, IS, and airway tissue samples, but also in EBC and BALF. EBC periostin levels and periostin tissue expression are higher in patients with asthma than in those with COPD. EBC periostin levels may serve as a potential surrogate marker for tissue periostin expression. Further studies on periostin in larger groups of patients with well-defined COPD and asthma phenotypes are required.

Contribution statement KG, PK, and RK designed the study. KG and MM-W were responsible for literature search, patient recruitment, data analysis, and drafting the manuscript. PK and RK performed bronchoscopy procedures. PN-G performed the cytological and periostin concentration measurements. MP-S conducted histological studies in bronchial biopsy specimens and was involved in data analysis and interpretation. All authors critically reviewed the manuscript and contributed to its final version.

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Analiza porównawcza ekspresji periostyny w różnych materiałach z dróg oddechowych u chorych na astmę i przewlekłą obturacyjną chorobę płuc

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astma, eozynofile, periostyna, przewlekła obturacyjna choroba płuc, zapalenie w drogach oddechowych

STRESZCZENIE

WPROWADZENIE Periostyna uważana jest za marker eozynofilowego zapalenia u chorych na astmę. W piśmiennictwie nie ma natomiast danych dotyczących znaczenia periostyny u chorych na przewlekłą obturacyjną chorobę płuc (POChP).

CELE Celem pracy była analiza ekspresji periostyny oraz porównanie jej stężenia w różnych materiałach u chorych na łagodną i umiarkowaną astmę i POChP, a także ocena potencjalnego związku między periostyną a klinicznymi cechami obu chorób.

PACJENCI I METODY Za pomocą testu immunoenzymatycznego oceniano stężenie periostyny w surowicy, plwocinie indukowanej (*induced sputum* – IS), kondensacie powietrza wydychanego (*exhaled breath condensate* – EBC) i płynie z płukania oskrzelowo-pęcherzykowego (*bronchoalveolar lavage fluid* – BALF) oraz ekspresję periostyny w bioptatach z oskrzeli u 24 astmatyków, 36 chorych na POChP i 12 osób z grupy kontrolnej. Dokonano również analizy korelacji między stężeniami periostyny w różnych materiałach oraz porównano jej stężenia u chorych na astmę i POChP.

WYNIKI Stężenia periostyny były wykrywalne w surowicy, IS, EBC i BALF zarówno u chorych na astmę i POChP, jak i w grupie kontrolnej. Stężenie periostyny w EBC było istotnie wyższe u chorych na astmę w porównaniu z chorymi na POChP ($p = 0,04$). Podobnie ekspresja tkankowa periostyny była wyższa u chorych na astmę niż u chorych na POChP ($p < 0,001$), jak również u chorych na astmę i POChP w porównaniu z grupą kontrolną ($p < 0,001$). Stwierdzono korelację między ekspresją periostyny w błonie śluzowej oskrzeli i jej stężeniem w EBC. Nie wykazano istotnej korelacji pomiędzy ekspresją tkankową periostyny i jej stężeniem w BALF, IS czy surowicy. Nie stwierdzono różnic w stężeniach periostyny w surowicy, IS, BAL lub EBC u chorych z różnymi fenotypami obu chorób.

WNIOSKI Periostyna może być wykrywalna nie tylko w surowicy, IS i bioptatach oskrzeli, ale także w EBC i BALF. Stężenie periostyny w EBC i jej ekspresja tkankowa są wyższe u chorych na astmę niż u chorych na POChP. Stężenie periostyny w EBC może służyć jako potencjalny marker zastępczy dla jej ekspresji tkankowej.

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