

ORIGINAL ARTICLE

Effect of treatment with inhaled corticosteroid on serum periostin levels in asthma

MAKOTO HOSHINO,¹ JUNICHI OHTAWA² AND KENTA AKITSU²¹Division of Clinical Allergy, Department of Internal Medicine, and ²Department of Radiology, Atami Hospital, International University of Health and Welfare, Atami, Japan**ABSTRACT**

Objective: Periostin is a biomarker of eosinophilic airway inflammation and may contribute to airway remodeling in asthma. The anti-inflammatory activity of inhaled corticosteroids (ICS) for asthma control is widely recognized. The aim of this study was to assess the effects of ICS on serum periostin levels and its relationships to inflammation and airway geometry.

Methods: Forty-two healthy controls and 20 patients with steroid-naïve asthma before and after treatment with fluticasone propionate (800 µg/day for 16 weeks) were examined. Serum periostin, lung function and inflammatory cell counts in sputum were measured. Airway dimensions were determined by quantitative computed tomography (total area of the airway (Ao), wall area (WA), wall thickness (T) and percentage wall area (WA%)).

Results: Serum periostin concentrations were significantly higher in patients with asthma than in controls. Periostin levels were correlated with airway wall thickness and sputum eosinophilia and inversely correlated with airflow limitation in asthma. ICS significantly decreased serum periostin ($P < 0.01$), decreased WA corrected for body surface area (WA/BSA, $P < 0.05$), $T/\sqrt{BSA}$ ($P < 0.01$) and WA% ($P < 0.01$), reduced the percentage of sputum eosinophils ($P < 0.01$) and improved airflow limitation. The decrease in serum periostin levels was associated with an increased per cent predicted forced expiratory volume in 1 s ($r = -0.64$, $P < 0.01$), decreased WA/BSA ($r = 0.46$, $P < 0.05$) and decreased sputum eosinophils ($r = 0.71$, $P < 0.01$).

Conclusion: Serum periostin levels respond partially to ICS and may reflect a reduction in airway inflammation and wall thickening in asthma.

Clinical trial registration: UMIN000013127 at www.umin.ac.jp

Key words: airway dimension, asthma, inflammation, inhaled corticosteroid, periostin.

SUMMARY AT A GLANCE

Serum periostin is a biomarker of eosinophilic inflammation and remodeling in asthma, and a course of inhaled corticosteroid therapy reduces serum periostin levels and is associated with improvement of airflow limitation.

Abbreviations: Ai, luminal area; Ao, total area of the airway; AQLQ, Asthma Quality of Life Questionnaire; BSA, body surface area; CT, computed tomography; ELISA, enzyme-linked immunosorbent assay; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; ICS, inhaled corticosteroids; IgE, immunoglobulin E; IL, interleukin; QoL, quality of life; RB1, right upper lobe apical segmental bronchus; SD, standard deviation; T, absolute wall thickness; Th2, T-helper type 2; WA, wall area; WA%, percentage wall area.

INTRODUCTION

Chronic airway inflammation and remodeling are key features of bronchial asthma. The airway inflammation is characterized by infiltration of eosinophils, mast cells and T-helper type 2 (Th2) cytokines, such as interleukin (IL)-4, IL-5 and IL-13.^{1,2} Evaluation of airway inflammation is important for asthma control. Direct airway sampling through bronchial biopsy or induced sputum is difficult to repeat and often impractical in clinical settings, and systemic biomarkers of eosinophilic inflammation are desirable for assessing the activity of asthma.

Periostin is an extracellular matrix protein, and its expression is upregulated by IL-4 and IL-13 in airway epithelial cells,^{3,4} and it has been observed in the airway subepithelial layer in patients with asthma.⁵ Moreover, the levels of periostin expression correlate with the thickness of the epithelial basement membrane.⁴ Therefore, periostin may be a key molecule that links Th2 inflammation and remodeling in asthma.

Remodeling is triggered by uncontrolled inflammation and results in fixed airway narrowing. Airway wall thickening is a prominent feature of remodeling, and it has been evaluated non-invasively in asthma using CT.⁶⁻⁸ Inhaled corticosteroids (ICS) are widely

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recognized as controller therapy because they reduce the production of Th2 and other cytokines, and they have a potent anti-inflammatory effect on the airways.⁹

We hypothesized that, in steroid-naïve patients with asthma with symptoms and airflow limitation, an upregulated periostin may be associated not only with airway inflammation but also with airway remodeling. To test this hypothesis, serum concentrations of periostin from steroid-naïve patients were compared with those of age-matched control subjects, and the relationships between periostin levels and airway inflammation and airway dimensions were examined.

METHODS

Patients

Forty-two healthy subjects and 33 steroid-naïve patients with asthma treated only with short-acting β_2 -agonists as required were enrolled. All patients were newly diagnosed with asthma. Eight patients did not meet the entry criteria. Asthma was diagnosed according to the American Thoracic Society criteria¹⁰ as a clear history of relevant symptoms and documented reversible airway obstruction ($>12\%$ improvement in forced expiratory volume in 1 s (FEV₁) after inhalation of a short-acting β_2 -agonist, and/or methacholine provocation concentration causing a 20% fall in FEV₁ (PC₂₀) < 8 mg/mL. All subjects were non-smokers or ex-smokers. Exclusion criteria for all subjects included a history of smoking (more than 5 pack-years total or had not stopped smoking more than 12 months before study entry), malignancy or lung fibrosis as these might influence periostin levels.

Study design

After a pre-screening period (up to 7 days) and a 14-day run-in period, steroid-naïve patients with asthma underwent pulmonary function tests, CT scanning, sputum induction and blood sampling, and completed a quality of life (QoL) questionnaire.

Quality of life

The Asthma Quality of Life Questionnaire (AQLQ) contained 32 items covering four domains (symptoms, activities, emotions and environment) using a 7-point scale.¹¹ A change of > 0.5 point represents a clinically meaningful improvement in QoL.

Study medication

Subjects were treated with fluticasone propionate (Glaxo Smith Kline, London, UK) 400 μ g twice daily for 16 weeks. The severity of asthma was defined according to the Global Initiative for Asthma-based symptoms and lung function.¹² Asthma was classified as intermittent in 0 patients, mild persistent in 14, moderate persistent in 9 and severe persistent in 2. Although some of patients might have required at

least one more controller drugs (such as long-acting β_2 -agonists, leukotriene receptor antagonists) from the beginning, patients were given fluticasone propionate alone because the aim of the study was to evaluate the effects of ICS on serum periostin. During the treatment phase, patients visited the hospital every 4 weeks. At the end of the 16-week period, the patients returned to the hospital for a final evaluation. All subjects gave their written, informed consent, and the study protocol was approved by the hospital's ethics committee. The study was registered with umin.org (University Hospital Medical Information Network) as: UMIN000013127.

Measurement of periostin

Serum periostin concentrations were measured by an enzyme-linked immunosorbent assay (ELISA) with the DuoSet Human Periostin/OSF-2 (R&D Systems, Minneapolis, MN, USA). This ELISA kit is specific for the measurement of natural and recombinant human periostin. It has been tested on human periostin isoforms 1 and 2 and does cross-react with mouse periostin. Different samples containing human periostin were diluted several fold (1/50 to 1/100), and the measured recoveries ranged from 95–105%. Intra-observer error was tested by four randomly selected samples eight times, and inter-observer error was determined by four samples and four separate assays. The coefficients of variability were $7.12 \pm 1.74\%$ and $7.62 \pm 1.95\%$ (mean $\pm$ standard deviation (SD)), respectively.

Pulmonary function

Spirometry (forced vital capacity (FVC), FEV₁ and FEV₁/FVC ratio) was performed for all subjects using computed spirometry (Fukuda Denshi, Tokyo, Japan). The predicted values of FVC and FEV₁ for a Japanese population were calculated using the formula proposed by the Japanese Respiratory Society.¹³

CT measurements

Scans were performed at full inspiration using a 64 multi-slice scanner (Aquilion-64, Toshiba Medical, Tokyo, Japan), as described previously.^{8,14} Scan parameters were as follows: 120 kV; 200 mA; collimation, 0.5 mm; rotation time, 0.5 s; pitch, 0.83. Images were reconstructed using a bone algorithm at a slice thickness of 1 mm and an interval of 0.5 mm. The full width at half-maximum principle¹⁵ was applied to determine the following airway parameters: luminal area (Ai), total area of the airway (Ao), wall area (WA = Ao – Ai), absolute wall thickness (T), and percentage wall area (WA% = WA/Ao $\times$ 100) at the right upper lobe apical segmental bronchus (RB1). Since airway size may be affected by body size, airway wall area was normalized to body surface area (BSA). To assess changes in lung volume, the cross-sectional area of the lung was measured before and after treatment by tracing the outer perimeter of the lung parenchyma on the same slice that was used for airway measurement.¹⁶ Airway dimensions were measured by one observer (J.O.) in a blind fashion.

Intra-observer error was tested by having this observer measure Ai and WA% in 10 randomly selected subjects twice. Analysis of reproducibility was assessed by the Bland–Altman method. Details about data reproducibility are described elsewhere.⁸

Sputum induction

Sputum induction and processing were performed as previously described¹⁷ with a slight modification. Briefly, subjects inhaled a 5% hypertonic saline solution from an ultrasonic nebulizer for 15 min, and adequate plugs of sputum were separated from saliva. After treatment with 0.1% dithiothreitol and phosphate-buffered saline, slides were prepared by cytopspin and stained by the May–Grünwald–Giemsa method. Differential cell counts were expressed as the percentage of 400 non-squamous cells.

Statistical analysis

Data are given as means (SD) or medians (interquartile range [IQR]). Comparisons between two groups were performed using unpaired *t*-tests. The relationships between serum periostin levels and clinical indices were tested using Pearson correlation coefficients. Paired *t*-tests or Wilcoxon signed-rank tests were used to compare variables before and after treatment. Relationships between parameters were assessed using Pearson correlation coefficients. Comparisons of multiple parameters were performed using analysis of variance with the Bonferroni correction. A *P* value < 0.05 was considered significant. All data were analysed with StatView software (SAS Institute, Cary, NC, USA).

RESULTS

Subject characteristics

Twenty patients completed the study. Study participation was terminated in three subjects due to exacerbations of asthma requiring medications and respiratory tract infections, and two subjects did not return for CT scanning as they were lost to follow-up. Only two subjects with asthma and two healthy controls subjects had a history of smoking (3, 1, 2 and 4 pack-years, respectively), and none had smoked within the past year. The characteristics of the 42 healthy control subjects and 20 patients with steroid-naïve asthma are shown in Table 1. No significant differences in age, gender and body mass index were observed between the two groups.

Serum periostin

Serum concentrations of periostin were significantly higher in steroid-naïve patients with asthma than in control subjects (*P* < 0.01; Table 1).

Pulmonary function

Percent predicted FEV₁ (FEV₁ % predicted) and FEV₁/FVC were significantly lower in patients with asthma than in control subjects (*P* < 0.001; Table 1).

CT measurements

For indices of airway wall thickness, patients with asthma had significantly higher WA/BSA (*P* < 0.01), T/√BSA (*P* < 0.001) and WA% (*P* < 0.001) than control

Table 1 Clinical characteristics

	Control subjects	Steroid-naïve asthma patients
Subjects (n)	42	20
Age (yr)	48 (12)	51 (13)
Male/female	19/23	9/11
Body mass index (kg/m ²)	23.6 (3.6)	23.5 (4.0)
Duration (yr)		19 (17)
Serum IgE (IU/mL)	n.d.	779.2 (905.0)
Serum periostin (ng/mL)	25.0 (17.1–31.0)	36.7 (31.2–45.1)*
Pulmonary function		
FVC (% predicted)	107.2 (18.9)	102.9 (17.2)
FEV ₁ (% predicted)	104.7 (11.0)	85.1 (22.9)**
FEV ₁ /FVC (%)	82.5 (6.0)	69.4 (11.6)**
CT scan measurement		
WA/BSA (mm ² /m ²)	14.0 (11.4–15.5)	15.3 (14.1–18.6)*
T/√BSA (mm/m)	0.77 (0.69–0.86)	1.13 (0.95–1.19)**
WA (%)	62.5 (58.4–65.1)	66.7 (64.2–69.4)**
Ai/BSA (mm ² /m ²)	8.5 (6.7–10.9)	7.8 (6.4–10.4)
Sputum		
Eosinophils (%)	0.0 (0.0–5.0)	16.0 (7.7–40.2)**
Neutrophils (%)	24.8 (13.0–38.7)	29.0 (15.7–42.5)

P* < 0.01, *P* < 0.001, control versus steroid-naïve asthma.

Data are presented as mean (SD) or median (IQR).

Ai, luminal area; BSA, body surface area; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; IgE, immunoglobulin E; n.d., not done; T, absolute wall thickness; WA, airway wall area.

subjects (Table 1). There was no significant difference in Ai/BSA between the two groups.

Induced sputum

Sputum samples were obtained from 25 control subjects and 16 steroid-naïve patients with asthma. The percentage of eosinophils in induced sputum was significantly higher in patients with asthma than in control subjects ($P < 0.001$). There was no difference in the sputum neutrophil percentage between the two groups (Table 1).

Relation of serum periostin levels to clinical indices

Correlations between serum periostin levels and spirometry, airway dimensions, and sputum

Table 2 Relationships between serum periostin and clinical indices in steroid-naïve asthma patients

Variables	Serum periostin	
	<i>r</i> -value	<i>P</i> -value
FEV ₁ (% predicted)	-0.61	<0.01
FEV ₁ /FVC (%)	-0.56	<0.05
WA/BSA (mm ² /m ²)	0.77	<0.01
T/√BSA (mm/m)	0.83	<0.001
WA (%)	0.67	<0.01
Sputum eosinophils (%)	0.65	<0.01

BSA, body surface area; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; T, absolute wall thickness; WA, airway wall area.

eosinophils are shown in Table 2. Serum periostin levels were negatively correlated with FEV₁ % predicted ($r = -0.61$, $P < 0.01$) and FEV₁/VC ($r = -0.56$, $P < 0.05$) and positively correlated with WA/BSA ($r = 0.77$, $P < 0.01$), T/√BSA ($r = 0.83$, $P < 0.001$), WA% ($r = 0.67$, $P < 0.01$) and percentage of sputum eosinophils ($r = 0.65$, $P < 0.01$) in patients with steroid-naïve asthma.

Effects of treatment with ICS

ICS significantly decreased serum periostin levels. FEV₁ and FVC, both absolute values and per cent of predicted values, increased significantly after treatment. WA/BSA, T/√BSA and WA% decreased significantly, but Ai/BSA did not change after treatment. Lung area was not affected by treatment. The percentage of sputum eosinophils decreased significantly after treatment. Domain analysis of the AQLQ showed significant improvements in symptoms and activities after treatment (Table 3). The decrease in the serum periostin level was associated with increased FEV₁ % predicted ($r = -0.64$, $P < 0.01$; Fig. 1a) and decreased WA/BSA ($r = 0.46$, $P < 0.05$; Fig. 1b), T/√BSA ($r = 0.49$, $P < 0.05$), WA% ($r = 0.50$, $P < 0.05$) and percentage of sputum eosinophils ($r = 0.71$, $P < 0.01$; Fig. 1c), and changed in AQLQ scores of symptoms ($r = 0.52$, $P < 0.05$) and activities ($r = 0.48$, $P < 0.05$). The changes in FEV₁ was correlated with the changes in WA/BSA ($r = 0.79$, $P < 0.01$) and in sputum eosinophils ($r = 0.78$, $P < 0.01$; data not shown).

Table 3 Serum periostin, pulmonary function, airway dimensions and induced sputum in asthmatic patients before and after inhaled corticosteroid treatment

Variables	Before treatment	After treatment	<i>P</i> -value
Serum periostin (ng/mL)	36.7 (31.2–45.1)	27.6 (24.1–35.5)	0.004
Pulmonary function			
FEV ₁ (L)	2.31 (0.88)	2.57 (0.86)	0.031
FEV ₁ (% predicted)	85.1 (22.9)	96.2 (20.3)	0.020
FVC (L)	3.24 (0.89)	3.49 (0.96)	0.015
FVC (% predicted)	102.9 (17.2)	112.6 (15.9)	0.009
FEV ₁ /FVC (%)	69.4 (11.6)	73.4 (11.4)	0.074
CT scan measurement			
WA/BSA (mm ² /m ²)	15.3 (14.1–18.6)	14.8 (13.8–17.6)	0.039
T/√BSA (mm/m)	1.13 (0.95–1.19)	0.91 (0.80–1.13)	0.001
WA (%)	66.7 (64.2–69.4)	64.1 (62.1–67.5)	0.006
Ai/BSA (mm ² /m ²)	7.8 (6.4–10.4)	8.2 (6.4–10.2)	0.061
Lung area (cm ²)	84.4 (82.4–86.0)	86.1 (81.8–90.1)	0.340
Sputum			
Eosinophils (%)	16.0 (7.7–40.3)	6.0 (1.2–16.0)	0.003
Neutrophils (%)	29.0 (15.7–42.5)	27.4 (14.4–48.1)	0.120
AQLQ			
Symptoms	4.8 (1.4)	5.6 (1.5)	0.036
Activities	5.0 (1.3)	5.6 (1.3)	0.029
Emotions	4.8 (1.6)	5.5 (1.4)	0.066
Environment	5.1 (1.4)	5.5 (1.5)	0.134

Ai, luminal area; AQLQ, Asthma Quality of Life Questionnaire; BSA, body surface area; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; T, absolute wall thickness; WA, airway wall area.

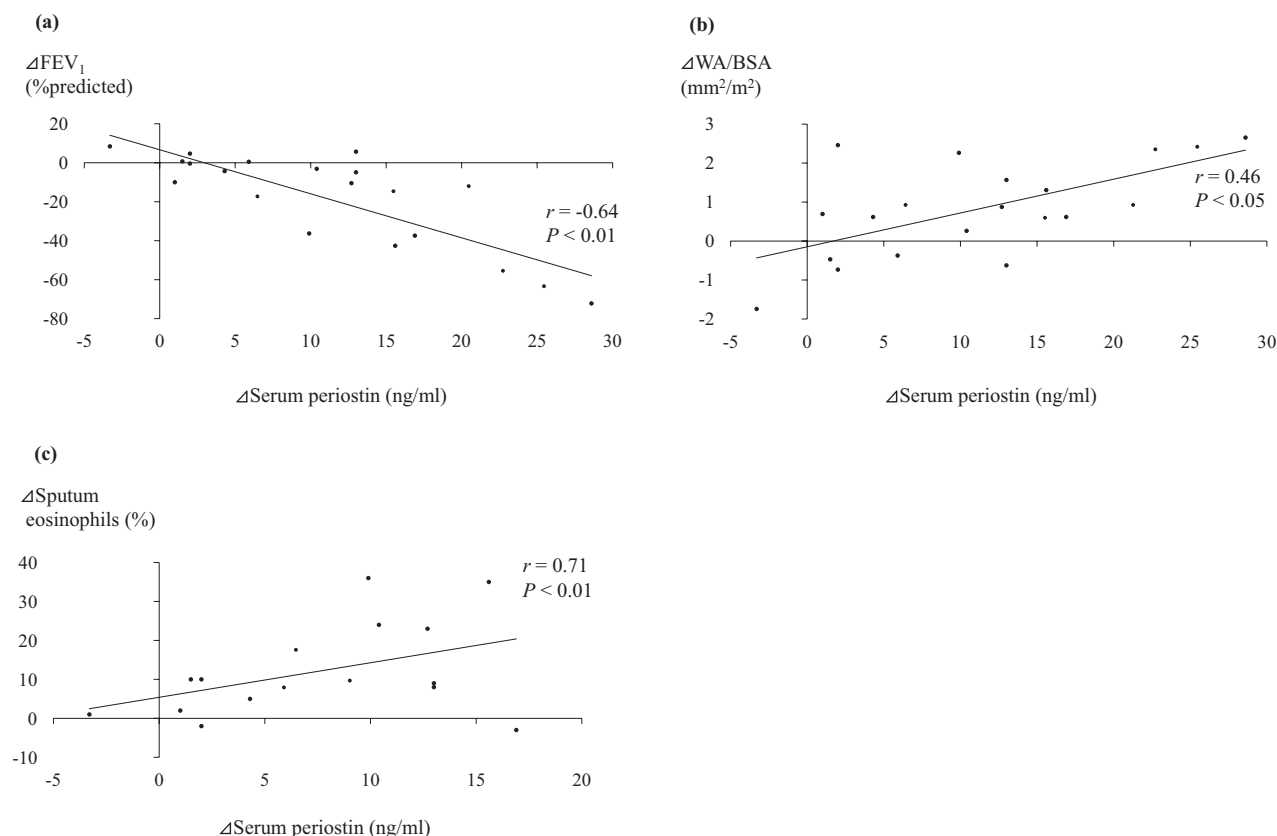


Figure 1 Relationships between changes in (a) serum periostin levels and FEV₁, and (b) serum periostin levels and wall area normalized to body surface area (WA/BSA), and (c) serum periostin and sputum eosinophils before and after inhaled corticosteroid treatment. BSA, body surface area; FEV₁, forced expiratory volume in 1 s; WA, wall area.

DISCUSSION

The present study found that serum periostin levels were significantly higher in patients with steroid-naïve asthma than in control subjects. Inhaled steroid therapy decreased the serum periostin levels, improved airflow limitation, reduced airway wall thickness and decreased sputum eosinophils in steroid-naïve patients with asthma. The reduction of serum periostin was associated with an increase in FEV₁ % predicted and decreases in WA/BSA and in sputum eosinophils. These results suggest that serum periostin could be a candidate for a novel marker for not only eosinophilic inflammation but as a biomarker for the development of airway remodeling.

Periostin is a secreted multicellular protein and that is highly expressed in bronchial epithelial cells and bronchial fibroblasts in asthmatic patients.^{3,5} The serum periostin concentration has been shown to be related to the number of eosinophils in bronchial tissue and sputum in patients with severe asthma.¹⁸ Secretion of periostin is upregulated by IL-4 and IL-13 stimulation, and serum periostin was identified as a reliable biomarker for airway eosinophilia in asthma. It is widely known that IL-4 and IL-13 are involved in subepithelial fibrosis,¹⁹ airway remodeling²⁰ and steroid responsiveness.²¹

Some patients with moderate-to-severe asthma have uncontrolled disease despite ICS therapy. These

patients with high serum periostin levels had greater improvement in lung function with lebrikizumab, an anti-IL-13 antibody, than those with low periostin levels.²² Taken together, periostin may be a key molecule that links remodeling and eosinophilic inflammation due to IL-13 in the asthmatic airway. In previous studies of steroid-naïve asthma patients, exacerbations of asthma contributed to airflow limitation, but ICS therapy improved pulmonary function, which might be interpreted to mean that the starting of corticosteroid therapy was associated with less risk of FEV₁ decline.²³ Kanemitsu *et al.* reported that high serum periostin concentrations were associated with a greater decline in FEV₁ in patients with asthma.²⁴ Serum periostin levels were higher in steroid-naïve asthma patients than in controls, which is in agreement with the findings of others that periostin reflects the development of airflow limitation.

In steroid-naïve asthma patients, ICS significantly reduced serum periostin levels, and this was associated with decreased wall thickness and a decreased percentage of sputum eosinophils. Moreover, the changes in serum periostin levels were correlated with improvement of FEV₁. The components of wall thickening that respond to steroid treatment may include the inflammatory process, as the decrease in wall thickness was correlated with the reduction of sputum eosinophils. Although not assessable by CT,

airway smooth muscle hypertrophy, basement membrane thickening, extracellular matrix deposition and increased cartilage might contribute to persistent airway wall thickness. Inhaled steroids may reduce the thickness of the reticular basement membrane,^{25–27} goblet cell hyperplasia²⁸ and the increased vascularity,²⁹ as shown by bronchial biopsy studies. However, ICS therapy cannot reduce extracellular matrix protein production by airway smooth muscle cells.³⁰ The luminal area in patients with asthma was similar to that in controls at baseline and did not change after treatment, even though airway wall thickness decreased. Previous studies^{7,31} have shown that Ai is not different between asthma and controls. The present finding that airway wall thickness (WA/BSA and T/√BSA) was related to the degree of airflow obstruction may be a distinctive index for airway remodeling in asthma. One study using CT showed that ICS reduces airway wall thickness, and this change was associated with increased FEV₁.³² Further studies will be needed to confirm these findings, although serum periostin levels may reflect chronic inflammation and remodeling of the asthmatic airways.

There were several limitations to the present study. First, this study did not include a placebo arm. Although placebo controls are increasingly difficult to justify in symptomatic asthma, lack of a placebo study should be considered when interpreting open-label data. Second, the small number of subjects could potentially affect the accuracy of the outcomes, especially the airway dimensions. To confirm the present findings, further studies with larger number of subjects should be performed. Third, only one bronchus was assessed; thus, the results of the current study might not be representative of all bronchi. Although some researchers have shown that RB1 measurement was closely related to measurements of other airways,^{8,31,33} airway dimensions may vary even in other bronchi. Fourth, CT images were obtained with a full inspiration breath hold on two different occasions, before and after treatment. CT images might not have been obtained at precisely the same lung volume at each session. However, lung area, which was not different because it is believed that total lung capacity remains constant during stable asthma, was measured.

In conclusion, serum periostin levels respond partially to treatment with ICS, and the decrease in serum periostin levels may reflect not only reduction in airway inflammation, but also airway wall thickness.

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