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Original article

Serum periostin is associated with body mass index and allergic rhinitis in healthy and asthmatic subjects

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ALT, alanine aminotransferase; AR, allergic rhinitis; AST, aspartate aminotransferase; BMI, Body mass index; BUN, blood urea nitrogen; Cre, creatinine; eGFR, estimate glomerular filtration rate; FEV₁, forced expiratory volume within 1 s; FPG, fasting plasma glucose; FVC, forced vital capacity; GGT, gamma-glutamyl transferase; HDL-C, high density lipoprotein cholesterol; IgE, immunoglobulin E; LDL-C, low density lipoprotein; NGSP, National Glycohemoglobin Standardization Program; TG, triglyceride; UA, uric acid

ABSTRACT

Background: Many studies have attempted to clarify the factors associated with serum periostin levels in asthmatic patients. However, these results were based on studies of subjects mainly characterized by high eosinophil counts, which may present as an obstacle for clarification in the identification of other factors associated with serum periostin levels. The aim of this study was to determine the factors associated with serum periostin levels in healthy subjects. We also assessed some factors in asthmatic subjects to confirm their extrapolation for management of asthma.

Methods: Serum periostin levels were measured in 230 healthy subjects. Clinical factors of interest included body mass index (BMI) and allergic rhinitis (AR). Additionally, we confirmed whether these factors were associated with serum periostin in 206 asthmatic subjects. We further evaluated several obesity-related parameters, such as abdominal fat distribution and adipocytokine levels.

Results: Smoking status, blood eosinophil count, total immunoglobulin E, and the presence of AR were associated with serum periostin in healthy subjects. There was a negative association between BMI and serum periostin in both healthy and asthmatic subjects, while there was a tendency of a positive association with AR in asthmatic subjects. There were no differential associations observed for subcutaneous and abdominal fat in relation to serum periostin in asthmatic subjects. Serum periostin was significantly associated with serum levels of adiponectin, but not with leptin.

Conclusions: Our results provided clarity as to the factors associated with serum periostin levels, which could be helpful in the interpretation of serum periostin levels in clinical practice.

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Introduction

Periostin is a secreted extracellular matrix protein that belongs to the fasciclin family. As a downstream molecule of interleukin (IL)-13, periostin has been proposed as a biomarker for the signature type-2 immune responses in asthmatic patients.^{1–4} Functionally, periostin is a regulator of fibrosis and collagen

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deposition and has been recognized for its vital role in airway remodeling.⁵ In this respect, high serum periostin levels have been associated with peripheral/airway eosinophilia, severe airflow limitation, and greater decline in forced expiratory volume (FEV) within 1 s (FEV₁) in asthmatic patients.^{3,4,6,7} In addition, serum periostin levels are associated with a favorable response to asthma treatment including novel biologics such as anti-immunoglobulin E (IgE) and IL-13 monoclonal antibodies, suggesting that serum periostin could be used as a surrogate maker of successful asthma treatment.^{8,9}

In addition to its role in asthma treatment, periostin has been found to be related to the physiopathology of numerous diseases, owing to its wide range of functions. For example, high levels of periostin expression have been detected in organs in certain diseases such as fatty liver disease, diabetes, and renal injuries.^{10–12} Studies using mice deficient in periostin and its neutralizing antibody have suggested its role in the pathogenesis of the aforementioned diseases.^{13,14} In addition, the local expression of periostin in the nasal mucosa of patients with allergic rhinitis (AR),¹⁵ which occurs in approximately 30% of Japanese adults, has been reported.^{16,17} These studies suggest that serum periostin levels are influenced by several factors related to common diseases such as metabolic disease, liver and renal impairment, and AR.

To date, numerous studies have attempted to clarify the clinical characteristics and phenotypes associated with elevated serum periostin levels in asthmatic patients.^{2–4,6} However, these studies were conducted in patients with asthma, which may confound one's ability to determine other factors associated with serum periostin levels. Considering the vast heterogeneity of eosinophilic inflammation in patients with asthma and the strong association between asthma and serum periostin levels, other factors that impact serum periostin levels among patients with asthma may have been masked in these studies. Therefore, it is necessary to investigate the factors associated with serum periostin in non-asthmatic subjects. Based on this concept, Caswell-Smith *et al.* have reported several factors associated with serum periostin levels in subjects without asthma or chronic obstructive pulmonary disease (COPD).¹⁸

We have conducted case–control studies to identify novel genetic factors and biomarkers of asthma have recruited subjects without pulmonary diseases or malignant diseases as controls for patients with asthma.^{19–21} In the present study, using these subjects, we first attempted to identify factors associated with serum periostin levels. We were particularly interested in the association between serum periostin levels and body mass index (BMI), and AR, both of which are significant comorbidities associated with asthma. Therefore, we also confirmed whether similar associations were found in asthmatic subjects to determine their clinical utility in the management of asthmatic subjects. In addition, in order to further clarify the relationship between obesity and periostin levels, we evaluated several obesity-related indices such as subcutaneous/abdominal fat density, as assessed by computed tomography (CT), and blood adipocytokine levels.

Methods

This study was approved by the Ethical Review Boards of Hokkaido University (14-057), Hokkaido University Hospital (009-0205), and JR Sapporo Hospital (2012-5). Written informed consent was directly obtained from study subjects by doctors.

Non-asthmatic subjects

Healthy Japanese subjects who underwent their annual health check at the JR Sapporo Hospital (Sapporo, Japan) were recruited

for enrollment into this study. Subjects were asked to answer the Japanese edition of the European Community Respiratory Health Survey (ECRHS) questionnaire,^{16,22,23} as summarized in the online supplement. Subjects who had any of the following conditions were excluded from the study: history of any pulmonary diseases or respiratory symptoms (Questions 1–6,8,10,11, part of 15 and 16); abnormal chest X-ray shadow, evaluated by respiratory physicians; percentage of predicted vital capacity (VC) < 80%; ratio of FEV₁ to forced vital capacity (FEV₁/FVC) < 70%; presence or past history of malignant diseases. The remaining subjects were enrolled in the study and classified as “non-asthmatic subjects”.

Asthmatic subjects

Recruitment of asthmatic subjects has been previously described.^{4,24} This population consisted of Japanese patients with asthma who had been enrolled in the Hokkaido-based Investigative Cohort Analysis for Refractory Asthma (Hi-CARAT). This study was registered in the University Hospital Medical Information Network (UMIN) Clinical Trials Registry system (UMIN ID 000003254) (https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr_view.cgi?recptno=R000003917). Diagnosis of severe asthma was based on the American Thoracic Society criteria for refractory asthma published in 2000²⁵, with slight modifications.^{4,24}

Smoking status

Patients were categorized into three groups: current continuous smokers (Current; Affirmative question, Do you now smoke, as of 1 month ago?), former smokers who had stopped smoking for a minimum of 1 month before the initial visit (Ex; Affirmative question of Have you ever smoked for as long as a year?), and lifetime non-smokers (Never) (Supplementary Methods). In addition, we evaluated cumulative smoking exposure by calculating pack-year (PY) by questions “How many cigarettes to you smoke every day?” “How many cigarettes had you being smoking?” (From years old to years old per day)” (Supplementary Methods).

Anthropometric measurements

Subjects were weighed wearing minimal clothes and without shoes; weight was rounded to the nearest 0.1 kg. Additionally, height was measured to the nearest 0.1 cm. BMI was calculated as weight (kg) divided by the squared height (m). A clinical research coordinator (CRC) or nurse measured the waist circumference (WC) at the navel of each subject in a standing position, rounding to the nearest 0.1 cm, using the methods proposed by the Japan Society for the Study of Obesity.²⁶

Definition of allergic rhinitis

Presence of rhinitis symptoms was assessed using questionnaires, according to the Practical Guideline for the Management of Allergic Rhinitis in Japan 2009.²⁷ Subjects were asked, “Do you have any nasal allergies including hay fever?” (Supplementary Methods).

Analysis of blood samples

Blood samples were collected for testing after fasting for at least 12 h. Peripheral blood eosinophil counts were obtained from standard complete blood counts, which were performed at the laboratory division of Hokkaido University Hospital. Total serum IgE levels (IU/mL) and IgE responses to commonly inhaled allergens, including *Dermatophagoides farinae*, grass pollens, animal dander, and molds, were determined. Atopy was defined on the

basis of multiple positive antigen simultaneous test scores (>1.01 lumicount) to at least one commonly inhaled allergen.⁴ For measurements of periostin, leptin, and adiponectin, serum samples were stored at -80°C until assay. Serum periostin levels were measured using an enzyme-linked immunosorbent assays (ELISA) at Shino-test (Kanagawa, Japan), as previously described.^{3,4,6} Leptin and adiponectin were measured using double-antibody radioimmunoassays and latex immune nephelometry, respectively (SRL, Japan). Other biochemistry markers were measured using standard methods at the JR Sapporo Hospital and Hokkaido University Hospital.

Abdominal fat CT imaging

Abdominal CT scans were performed with patients in the supine position by using a multidetector-row spiral CT scanner with a 64-detector array (Aquilion64; Toshiba Medical Systems, Otawara, Japan). Visceral fat area (VFA) and subcutaneous fat area (SFA) were calculated using the Fat Scan software program.²⁸

Statistical methods

Continuous data were presented as means and standard deviations (SD) or medians and interquartile ranges (IQR), while categorical data were presented as frequencies and proportions. To compare differences among the groups, Student's *t*-tests or one-way analysis of variance were used for parametric continuous variables, Mann–Whitney U tests were used for nonparametric continuous variables, and chi-square tests were used for categorical variables. To evaluate the correlation between two parameters, Pearson's correlation coefficient was calculated. We conducted multivariate analyses to determine factors significantly associated with serum periostin levels, while controlling for potential confounding factors. To analyze the tendency of continuous and categorical variables among the groups, we performed Jonckheere–Terpstra trend tests or Cochran–Armitage trend tests. Statistical analyses were performed using the statistical software package SYSTAT for Windows (version 13.1; SYSTAT, San Jose, CA, USA) and EZR (version 1.27; Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria).²⁹ For all statistical analyses, two-sided *P*-values <0.05 were considered statistically significant.

Results

Characteristics of non-asthmatic subjects

Table 1 and Supplementary Table 1 summarize the characteristics of 230 non-asthmatic subjects included in this study. In total, 143 (62.2%) were men with a mean age and BMI of 51.4 years and 22.8 kg/m^2 , respectively. Seventy-six (33.0%) and 99 (43.0%) subjects had AR symptoms and atopy, respectively. Sixty-nine (30.0%) subjects were current smokers, 53 (23.0%) were ex-smokers, and 108 (47.0%) were non-smokers. Median PY was 5.6 (IQR 0–28.0). Mean Geometric blood eosinophil count was $116.3\text{ cells}/\mu\text{L}$. Supplementary Tables 2–4 show the characteristics of non-asthmatic subjects stratified by the presence of AR and smoking status. The prevalence of atopy and total IgE levels were significantly higher in subjects with AR than those without AR ($P < 0.0001$ and $P < 0.01$, respectively). Additionally, PY in subjects with AR was significantly higher than in those without AR ($P < 0.0001$).

Table 1

Characteristics of non-asthmatic subjects (N = 230).

Male sex, N (%)	143 (62.2%)
Age, years	51.4 $\pm$ 7.3
Smoking status, N (%)	
Current smoker	69 (30.0%)
Ex-smoker	53 (23.0%)
Never smoker	108 (47.0%)
Pack years	5.6 (0–28.0)
Body mass index, kg/m^2	22.8 $\pm$ 3.0
Atopy, N (%)	99 (43.0%)
Symptom of allergic rhinitis, N (%)	76 (33.0%)
FEV ₁ , % predicted (pre-bronchodilator)	105.6 $\pm$ 13.0
FEV ₁ /FVC, % (pre-bronchodilator)	82.4 $\pm$ 5.9

FEV₁, forced expiratory volume within 1 s; FVC, forced vital capacity.

Data are shown as mean $\pm$ SD, median (IQR) or number (%).

Distribution of serum periostin levels

Distribution of serum periostin levels and logarithm-transformed periostin (log periostin) are presented in Supplementary Fig. 1. The mean geometric value of periostin level was 66.1 ng/mL . The minimum and maximum levels were 30.0 and 155.0 ng/mL , respectively.

Factors associated with serum periostin levels in non-asthmatic subjects

Female subjects had higher levels of serum periostin when compared with male subjects (69.8 vs. 64.0 ng/mL , $P = 0.04$). Serum periostin levels significantly differed according to smoking status (Non-smokers, 69.4 ; ex-smoker 66.6 ; current smokers, 61.0 ng/mL ; $P = 0.027$) (Supplementary Table 3). PY was negatively associated with serum periostin levels ($PY < 10$, 69.8 ; $PY \geq 10$, 62.1 ng/mL ; $P = 0.0048$) (Supplementary Table 4). Blood eosinophil counts and total IgE were significantly associated with serum periostin ($R = 0.148$, $P = 0.025$; $R = 0.150$, $P = 0.023$, respectively). Alanine aminotransferase (ALT; $R = -0.175$, $P = 0.0077$), gamma-glutamyl transpeptidase (γ -GTP; $R = -0.208$, $P = 0.0015$), and uric acid (UA; $R = -0.158$, $P = 0.016$) were significantly associated with serum periostin levels. Serum periostin levels were significantly and inversely associated with BMI ($R = -0.165$, $P = 0.012$) (Table 2, Supplementary Fig. 2A); this trend remained even after adjustment for age and sex ($P = 0.057$) (Supplementary Table 5). Subjects with AR symptoms exhibited higher serum periostin levels compared with those without AR symptoms (73.0 vs. 63.0 ng/mL , $P = 0.0007$). This remained significant even after adjustment for confounding factors ($P = 0.0057$) (Supplementary Table 2).

Characteristics of asthmatic subjects and factors associated with serum periostin levels

Table 3 summarizes the characteristics of asthmatic subjects in this study, which are identical with those in our previous report.⁴ Of the 206 subjects, 79 (38.3%) subjects had mild-to-moderate asthma and 127 (61.7%) had severe asthma. Eighty-three (40.3%) subjects were men with a mean age of 59.5 years and a mean BMI of 25.0 kg/m^2 . Geometric mean blood eosinophil and total serum IgE were $214.6\text{ cells}/\mu\text{L}$ and 146.6 IU/mL , respectively. One hundred fifty-six subjects (75.7%) had AR (Table 3).

Total IgE and blood eosinophil counts were significantly associated with serum periostin levels (IgE: $R = 0.231$, $P = 0.0009$; eosinophil count: $R = 0.437$, $P < 0.0001$) (Table 4). Similar to the findings among non-asthmatic subjects, BMI was significantly and inversely associated with serum periostin levels ($R = -0.178$,

Table 2
Correlations with serum periostin levels in non-asthmatic subjects (N = 230).

	R	P-value
Age, years	0.140	0.034
Body mass index, kg/m ²	−0.165	0.012
Pack years [†]	−0.199	0.0024
Blood eosinophils, cells/ μ L [‡]	0.148	0.025
Blood neutrophils, cells/ μ L [‡]	−0.118	0.074
Serum total IgE, IU/mL [‡]	0.150	0.023
AST, U/L [‡]	−0.102	0.125
ALT, U/L [‡]	−0.175	0.0077
GGT, U/L [‡]	−0.208	0.0015
BUN, mg/dL [‡]	0.0091	0.890
Cre, mg/dL [‡]	−0.058	0.383
eGFR, mL/min/1.73 m ² [‡]	−0.060	0.366
UA, mg/dL [‡]	−0.158	0.016
TG, mg/dL [‡]	−0.085	0.200
HDL-C, mg/dL [‡]	0.065	0.325
LDL-C, mg/dL [‡]	0.042	0.525
FPG, mg/dL [‡]	−0.112	0.089
HbA1c (NGSP), % [‡]	−0.054	0.414

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Cre, creatinine; eGFR, estimate glomerular filtration rate; FPG, fasting plasma glucose; GGT, gamma-glutamyl transferase; HDL-C, high density lipoprotein cholesterol; IgE, immunoglobulin E; LDL-C, low density lipoprotein; NGSP, National Glycohemoglobin Standardization Program; TG, triglyceride; UA, uric acid.

Pearson's correlation coefficient unless otherwise stated.

[†] Spearman's rank correlation coefficient.

[‡] Log-transformed.

Table 3
Characteristics of asthmatic subjects (N = 206).

Male sex, N (%)	83 (40.3%)
Age, years	59.5 $\pm$ 13.8
Severity of asthma (mild to moderate/severe)	79/127
Smoking status, N (%)	
Current smoker	21 (10.2%)
Ex-smoker	97 (47.1%)
Never smoker	88 (42.7%)
Pack years	3.3 (0–18.8)
Body mass index, kg/m ²	25.0 $\pm$ 5.0
Atopy, N (%)	140 (68.0%)
Symptom of allergic rhinitis, N (%)	156 (75.7%)
Blood eosinophils, %	4.0 (1.8–7.0)
Blood eosinophils, cells/ μ L	214.6 (0.44)
Blood neutrophils, %	62.0 (54.9–69.5)
Blood neutrophils, cells/ μ L	3910.4 (0.19)
Serum total IgE, IU/mL	146.6 (0.72)
Serum periostin, ng/mL	85.1 (0.19)
FEV ₁ , % predicted (pre-bronchodilator)	95.4 $\pm$ 19.1
FEV ₁ /FVC, % (pre-bronchodilator)	68.5 $\pm$ 12.5

AR, allergic rhinitis; IgE, immunoglobulin E; FEV₁, forced expiratory volume within 1 s; FVC, forced vital capacity.

Data are shown as mean $\pm$ SD, median (IQR), or number (%).

Table 4
Factors correlated with serum periostin levels in asthmatic subjects (N = 206).

	R	P-value
Age, years	0.105	0.133
Body mass index, kg/m ²	−0.178	0.010
Pack years [†]	−0.136	0.051
% predicted FEV ₁	−0.140	0.045
Blood eosinophils, cells/ μ L [‡]	0.437	<0.0001
Blood neutrophils, cells/ μ L [‡]	−0.109	0.117
Serum total IgE, IU/mL [‡]	0.231	0.00085

FEV₁, forced expiratory volume within 1 s; IgE, immunoglobulin E.

Pearson's correlation coefficient unless otherwise stated.

[†] Spearman's rank correlation coefficient.

[‡] Log-transformed.

P = 0.010, Table 4, Supplementary Fig. 2B). Furthermore, the association between serum periostin and BMI remained significant after adjustment for several clinical factors (P = 0.020; Supplementary Table 5). Serum periostin levels of the subjects with AR were higher than in those without AR, although this relationship was not significant (P = 0.173; Supplementary Table 6).

Association between serum periostin levels and AR for all patients

When all subjects were stratified according to the presence or absence of asthma and AR, a significant trend was observed in the increase in serum periostin levels among the four groups (P < 0.0001; Fig. 1).

Association between serum periostin levels with obesity-related indices

As BMI was negatively associated with serum periostin levels in both non-asthmatic and asthmatic subjects, we further evaluated the associations with other obesity-related parameters (Table 5). Both in non-asthmatic and asthmatic subjects, serum periostin was negatively associated with waist circumference (WC) (R = −0.143,

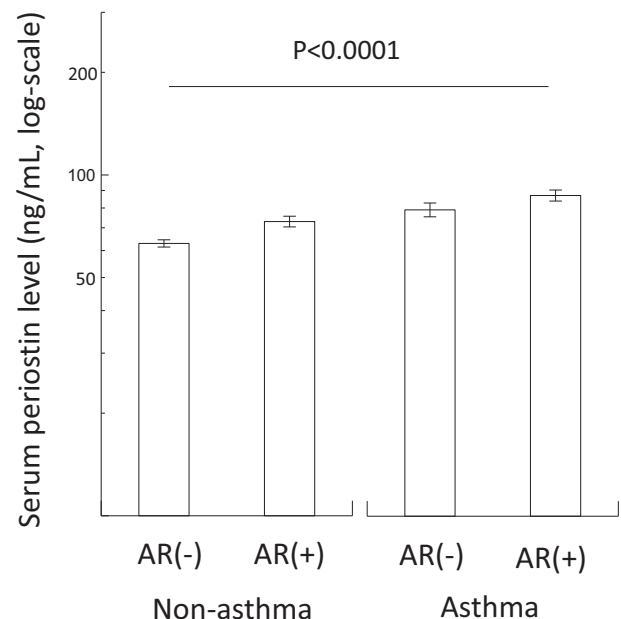


Fig. 1. Serum periostin levels according to the presence and absence of asthma and rhinitis. Jonckheere-Terpstra trend test showed significant increasing trend (P < 0.0001) of serum periostin levels among four groups. AR, allergic rhinitis.

Table 5
Correlation of obesity-related indices with serum periostin levels for non-asthmatic and asthmatic subjects.

	Non-asthmatic subjects		Asthmatic subjects	
	R	P-value	R	P-value
Waist circumference, cm	−0.143	0.031	−0.157	0.024
Leptin, ng/mL [†]	0.100	0.156	−0.200	0.004
Adiponectin, μ g/mL [†]	0.316	<0.0001	0.139	0.046
Subcutaneous fat			−0.197	0.005
Visceral fat			−0.161	0.021

Pearson's correlation coefficient unless otherwise stated.

[†] Log-transformed.

$P = 0.031$ in non-asthmatic subjects; $R = -0.157$, $P = 0.024$ in asthmatic subjects). Moreover, serum leptin was positively associated with periostin in asthmatic subjects ($R = -0.200$, $P = 0.004$) but not in non-asthmatic subjects. Furthermore, serum adiponectin was negatively associated with periostin both in non-asthmatic and asthmatic subjects ($R = 0.316$, $P < 0.0001$ in non-asthmatic subjects; $R = 0.139$, $P = 0.046$ in asthmatic subjects). In asthmatic subjects, both SFA and VFA, assessed using CT, were negatively associated with periostin ($R = -0.197$, $P = 0.005$ for subcutaneous fat; $R = -0.161$, $P = 0.021$ for visceral fat) (Table 5).

Discussion

In the present study, we initially attempted to identify clinical factors associated with serum periostin levels in subjects without any pulmonary diseases, including asthma. In addition to high expression in the lower airway of patients with asthma, one study has reported local expression of periostin in the nasal mucosa of patients with AR.¹⁵ Asthma and AR often co-exist, and it is believed that they represent a continuum, sharing common pathological and/or physiological characteristics in the upper and lower airways.^{16,17,30,31} These characteristics suggest that the presence of AR would also contribute to an increase in serum periostin levels in patients with asthma. In the present study, it is noteworthy that among non-asthmatic subjects, periostin levels were significantly higher in patients with AR than in those without AR. This association remained significant after adjustment for blood eosinophil counts, suggesting that the presence of AR itself perhaps contributes to an increase in serum periostin levels. In addition, a significant trend was observed in the increase of serum periostin levels among non-asthmatic and asthmatic subjects categorized according to the presence and absence of AR, also emphasizing the effects of AR on serum periostin levels. In cases where asthma and AR co-exist, the contribution of both AR and asthma must be considered when interpreting serum periostin levels. Although two studies have previously reported no impact of AR on serum periostin levels in children,^{32,33} this might be explained by the difference in the age of the population age, as serum periostin levels are highly influenced by body growth.³⁴

In this study, we also demonstrated that there was a negative association between BMI and serum periostin in non-asthmatic subjects and this trend was also observed in asthmatic subjects. Numerous previous studies, including unbiased clustering analyses, have determined obesity to be one of the distinct asthma phenotypes.^{16,17,35,36} Several mechanisms have been postulated as potential explanations for the impact of obesity on the asthma severity, including abnormal respiratory mechanics, bronchial hyper-responsiveness, and increased prevalence of obesity-associated comorbidities.^{37–40} Studies have also suggested that obesity alters the nature of airway inflammation, although such results have been inconsistent; obesity is associated with neutrophilic inflammation,^{35,41} however some studies have demonstrated that it enhances eosinophilic inflammation.^{42,43} In either case, given the marked influence of obesity on asthma severity, the results of the present study, which indicate that BMI is negatively associated with serum periostin in patients with asthma, are of significance when interpreting serum periostin levels in obese patients with asthma. On the basis of our results, serum periostin levels might be underestimated in obese patients with asthma when the degree of eosinophilic airway inflammation is similar to that observed in non-obese subjects. More importantly, the predictive value of serum periostin in relation to the efficacy of several biologics targeting Th2-related

molecules^{8,9} may also be underestimated in obese subjects, thus subject with need for these agents may be missed.

We further evaluated several obesity-related parameters such as abdominal fat distribution and blood adipocytokine levels in order to flesh out the relationship between BMI and periostin levels. However, we could not accurately determine or differentiate the associations with subcutaneous and abdominal fat. Of note, periostin was significantly associated with levels of serum adiponectin, but not with leptin, in healthy subjects. These findings might provide guidance as to the mechanisms driving the association between serum periostin levels and obesity.

The high expression of periostin in the liver of a murine model of hepatosteatosis in patients with fatty liver disease, and a marked lower degree of steatosis and inflammation and fibrosis in periostin-deficient mice compared with wild-type mice,^{13,14} suggesting that periostin could be a potential marker and/or a therapeutic target for hepatosteatosis. At the beginning of this study, we anticipated that serum periostin levels would be positively associated with several blood makers related to metabolic diseases; thus, the negative associations observed in the present study were unexpected. Additional studies are still warranted to clarify the exact role of periostin in the pathogenesis of several metabolic-related diseases.

Notably, serum periostin levels were significantly lower in smoking subjects compared with those in nonsmoking subjects. In contrast, the levels of other Th2-related biomarkers, such as blood eosinophils and serum total IgE, were higher in smoking subjects than in those in nonsmoking subjects. Thus, the smoking status should be considered for accurate evaluation of each Th2-related biomarker in clinical practice.

Several ELISA platforms are currently available, which might explain the difference of affinities of monoclonal antibodies for periostin isoforms.^{2,44,45} In this study, serum periostin levels were determined using an assay “Shino-test” (Kanagawa, Japan), which has been reported in several studies.^{3,4,6} An assay “Elecysys®” (Penzberg, Germany), has been also used in several studies,^{9,46} including that from Caswell-Smith *et al.*, which conducted similar approach to our study to identify the factors associated with serum periostin level in healthy subjects.¹⁸ In their study,¹⁸ the mean (SD) periostin level was 51.2 (11.9) ng/mL, which is lower than those of healthy subjects in our population (66.1 [0.14] ng/mL). However, James *et al.* have shown a strong correlation between the two assays.⁴⁷

We would like to emphasize that the aim of the present study was to determine factors associated with serum periostin levels, but not to compare levels between subjects with asthma and non-asthmatic subjects. In this study, for non-asthmatic subjects, we strictly excluded subjects with presence of or history of asthma using an ECRHS questionnaire, which has been used to evaluate the prevalence of asthma worldwide.^{22,23} In addition, subjects with any kind of respiratory or malignant diseases were also excluded. Meanwhile, patients with asthma were those already recruited in our ongoing cohort study, for which over half of whom have severe asthma and require high doses of inhaled corticosteroid (ICS) or oral corticosteroid (OCS) to achieve asthma control.⁴ Thus, both subjects with asthma and non-asthmatic subjects were not representative of actual populations, and the clinical characteristics of these two populations with regard to sex, age, and smoking status were not matched between the two populations. Therefore, it might be considered that the populations used were not entirely suitable for comparing serum periostin levels between the two groups or for determining the cut-off levels and sensitivity/specificity for differentiating patients with asthma.

A previous study proposed a periostin cut-off level of 95 ng/mL based on values obtained from 66 controls⁶; however, our results

indicate that the definition of healthy subjects would greatly influence the range of periostin level; thus, additional studies with a larger sample size are needed to define the exact periostin cut-off levels in each study. In addition, when interpreting comparisons between our results and those reported by Caswell-Smith *et al.*,¹⁸ as well as differences between the assays used, differences in the definitions used to categorize healthy subjects must be also considered. One strength of our study was that we carefully and strictly excluded subjects with pulmonary diseases using detailed questionnaires, pulmonary function tests, chest X-ray findings, and by direct asking by doctors for non-asthmatic subjects. However, we were not able to ascertain information regarding the presence of several cardiovascular diseases, chronic sinusitis, and/or nasal polyps. In addition, we did not perform chest CT and, thus, could not exclude subjects with mild emphysema even if they did not fulfill criteria for COPD (<70% FEV₁/FVC).

In conclusion, results from the present study which included both non-asthmatic and asthmatic subjects, determined the factors associated with serum periostin levels. The presence of AR was associated with an increase in serum periostin levels, whereas BMI was negatively associated with periostin levels. This information could be helpful in the interpretation of serum periostin levels in clinical practice and provides further insights regarding the role of periostin in humans.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.alit.2017.11.006>.

Conflict of interest

KI received research funding from Shino-Test and Chugai Pharmaceutical, and honorarium for his adviser's role from Chugai Pharmaceutical. MN received research funding from AstraZeneca, Kyorin Pharmaceutical and MSD. The rest of the authors have no conflict of interest.

Authors' contributions

HirokaK, statistical analysis, acquisition of data, interpretation of data, and drafting the manuscript; SK, study concept and design, acquisition of data, interpretation of data, and drafting the manuscript; HM, study concept and design, interpretation of data, and critical revision of the manuscript; NT study concept and design, acquisition of data, interpretation of data, and critical revision of the manuscript; HirokiK, HG, KaoS, NS, KatS acquisition of data and interpretation of data, and critical revision of the manuscript; MS, interpretation of data; JO, KI, measuring periostin level, interpretation of data, and critical revision of the manuscript; YMI, statistical analysis and critical revision of the manuscript; MN, study concept and design, interpretation of data, and finalizing of the manuscript.

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Supplementary Methods

Questionnaire used in this study (translated into English)

Please circle the correct choice

Ex: a Yes b No

What is today's date? _____ Year _____ Month _____ Day

What is your date of birth?

_____ / _____ (Month/Year) _____ years old

Are you male or female?

a Male b Female

1. Have you had wheezing or whistling in your chest at any time in the last 12 months?

a Yes b No

↳ GO TO QUESTION 2

1-1. Have you been at all breathless when the wheezing noise was present?

a Yes b No

1-2. Have you had this wheezing or whistling when you did not have a cold?

a Yes b No

2. Have you woken up with a feeling of tightness in your chest at any time in the last 12 months?

a Yes b No

3. Have you been woken by an attack of shortness of breath at any time in the last 12 months?

a Yes b No

4. Have you been woken by an attack of coughing at any time in the last 12 months?

- a Yes b No

5. Have you ever had asthma?

- a Yes b No



GO TO QUESTION 7

5-1. Was this confirmed by a doctor?

- a Yes b No

5-2. How old were you when you had your first attack of asthma?

_____ years old

5-3. How many attacks of asthma have you had in the last 12 months?

6. Are you currently taking any medicines (including inhalers, aerosols or tablets) for asthma?

- a Yes b No

7. Do you have any nasal allergies, including hay fever?

- a Yes b No



GO TO QUESTION 8

7-1. How old were you when you had your first symptoms of nasal allergies?

_____ years old

8. Have you had coughing and phlegm on most days for a minimum of 3 months a year and for at least 2 successive years?

- a Yes b No

9. Have you ever smoked for as long as a year?

a Yes

b No



GO TO QUESTION 8

9-1. When did you start smoking?

_____ years old

9-2. Do you now smoke, as of one month ago?

a Yes

b No



GO TO QUESTION 9-3

9-2-1. How many cigarettes do you smoke every day?



GO TO QUESTION 10

9-3. How many cigarettes had you being smoking?

From _____ years old to _____ years old _____ per day

10. Do you have difficulty in breathing on exertion in daily life?

a Yes

b No

11. Have you ever been diagnosed with emphysema or chronic bronchitis or chronic obstructive pulmonary disease (COPD)?

a Yes

b No

12. Have you ever had herpes zoster?

a Yes _____ years old

b No

13. Do you have any pets?

a Yes

b No



Type of animal _____ Since when _____

14. Hometown

1 Hokkaido 2 Outside of Hokkaido

15. Check the diseases you are currently suffering from?

☐atopic dermatitis ☐food allergy ☐sarcoidosis ☐tuberculosis☐non-tuberculosis *Mycobacterium* ☐hypertention ☐diabetes☐malignant disease (organ) ☐others ()

16. Check the diseases you had been suffered before.

☐asthma ☐hay fever ☐allergic rhinitis ☐atopic dermatitis ☐food allergy☐tuberculosis ☐non-tuberculosis *Mycobacterium*☐others ()

17. Check the diseases your family have.

☐asthma (who?) ☐hay fever () ☐nasal allergy ()☐atopic dermatitis () ☐sarcoidosis () ☐tuberculosis ()☐non-tuberculosis *Mycobacterium* () ☐others ()

Supplementary Table 1. Blood biomarkers in non-asthmatic subjects (N=230).

Blood eosinophils, %	2.4 (1.5-3.8)
Blood eosinophils, cells/ μ L	116.3 (0.37)
Blood neutrophils, %	57.0 (51.5-62.9)
Blood neutrophils, cells/ μ L	2824.4 (0.17)
Serum total IgE, IU/mL	46.6 (0.61)
Serum periostin, ng/mL	66.1 (0.14)
AST, U/L	24.6 (0.17)
ALT, U/L	23.2 (0.26)
GGT, U/L	33.9 (0.38)
BUN, mg/dL	13.8 (0.11)
Cre, mg/dL	0.76 (0.087)
eGFR, mL/min/1.73m ²	71.7 (0.074)
UA, mg/dL	5.5 (0.12)
TG, mg/dL	105.3 (0.25)
HDL-C, mg/dL	58.9 (0.11)
LDL-C, mg/dL	119.6 (0.11)
FPG, mg/dL	100.1 (0.060)
HbA1c (NGSP), %	5.8 (0.039)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Cre, creatinine; eGFR, estimate glomerular filtration rate; FPG, fasting plasma glucose; GGT, gamma-glutamyl transferase; HDL-C, high density lipoprotein cholesterol; IgE, immunoglobulin E; LDL-C, low density lipoprotein ; NGSP, National Glycohemoglobin Standardization Program; TG, triglyceride; UA, uric acid.

Data are shown as median (IQR) or geometric mean (log₁₀ SD).

Supplementary Table 2. Clinical characteristics of non-asthmatic subjects with or without allergic rhinitis.

	non AR (N=154)	AR (N=76)	P-value
Age, years	51.7±7.3	50.8±7.4	0.388
Smoking status, N (%)			0.0003
Current smoker	56 (36.4%)	13 (17.1%)	
Ex-smoker	40 (26.0%)	13 (17.1%)	
Never smoker	58 (37.7%)	50 (65.8%)	
Pack years	12.3 (0-32.0)	0 (0-12.8)	<0.0001
Body mass index, kg/m ²	23.2±3.0	21.8±2.8	0.0009
Atopy, N (%)	40 (26.0%)	59 (77.6%)	<0.0001
Blood eosinophils, cells/μL [†]	108.5 (0.35)	133.7 (0.40)	0.079
Serum total IgE, IU/mL [†]	37.7 (0.57)	71.6 (0.64)	0.0010
Serum periostin, ng/mL [†]	63.0 (0.13)	73.0 (0.14)	
Crude			0.0007
Adjusted [‡]			0.0057
Adjusted 2 [§]			0.031
FEV ₁ , % predicted	105.6±12.6	105.5±13.7	0.951
FEV ₁ /FVC, %	82.5±6.0	82.2±5.7	0.691

AR, allergic rhinitis; IgE, immunoglobulin E; FEV₁, forced expiratory volume within one second; FVC, forced vital capacity.

Data are shown as mean±SD, median (IQR), or number (%).

P-values were obtained by chi-square test, t-test, or Mann-Whitney U-test as appropriate

[†] Log-transformed.

[‡] Adjusted for age, sex, atopy, BMI and blood eosinophil.

[§] Adjusted for age, sex, serum IgE, BMI and blood eosinophil.

Supplementary Table 3. Clinical characteristics of non-asthmatic subjects according to smoking status (never, ex, current).

	Never smoked (N=108)	Ex-smoker (N=53)	Current-smoker (N=69)	P-value
Age, years	50.9±8.2	52.8±6.2	51.1±6.6	0.254
Pack years	0 (0-0)	20.0 (12.0-30.5)	31.0 (21.0-36.5)	<0.0001
Body mass index, kg/m ²	22.0±2.9	23.6±2.6	23.3±3.1	0.0017
Atopy, N (%)	59 (54.6%)	22 (41.5%)	18 (26.1%)	0.0009
Blood eosinophils, cells/μL [†]	106.9 (0.42)	100.5 (0.31)	148.5 (0.29)	0.014
Serum total IgE, IU/mL [†]	42.2 (0.66)	49.1 (0.52)	52.2 (0.59)	0.585
Serum periostin, ng/mL [†]	69.4 (0.13)	66.6 (0.15)	61.0 (0.13)	
Crude				0.027
Adjusted [‡]				0.063
Trend test [§]				0.072
FEV ₁ , % predicted	105.6±13.9	107.9±11.6	103.8±12.4	0.221
FEV ₁ /FVC, %	83.7±6.1	82.5±5.2	80.2±5.6	0.0007

IgE, immunoglobulin E; FEV₁, forced expiratory volume within one second; FVC, forced vital capacity.

Data are shown as mean±SD, median (IQR), or number (%).

P-values were obtained by chi-square test, one-way ANOVA, or Mann-Whitney U-test as appropriate.

[†] Log-transformed.

[‡] Adjusted for age, sex, atopy, body mass index, and blood eosinophil.

[§] Jonckheere-Terpstra trend test.

Supplementary Table 4. Clinical characteristics of non-asthmatic subjects according to smoking status based on the number of pack-years.

	PY<10 (N=123)	PY≥10 (N=107)	P-value
Age, years	50.6±8.0	52.2±6.3	0.096
Smoking status, N (%)			<0.0001
Current smoker	5 (4.1%)	64 (59.8%)	
Ex-smoker	10 (8.1%)	43 (40.2%)	
Never smoker	108 (87.8%)	0 (0%)	
Pack years	0 (0-0)	29.0 (20.3-36.8)	<0.0001
Body mass index, kg/m ²	22.0±2.8	23.7±2.9	<0.0001
Atopy, N (%)	64 (52.0%)	35 (32.7%)	0.003
Blood eosinophils, cells/μL [†]	106.1 (0.41)	129.2 (0.30)	0.078
Serum total IgE, IU/mL [†]	42.7 (0.64)	52.9 (0.56)	0.198
Serum periostin, ng/mL [†]	69.8 (0.13)	62.1 (0.14)	
Crude			0.0048
Adjusted [‡]			0.032
FEV ₁ , % predicted	105.7±13.6	105.5±12.3	0.895
FEV ₁ /FVC, %	83.7±6.0	80.8±5.4	0.0002

PY, pack-years; IgE, immunoglobulin E; FEV₁, forced expiratory volume within one second; FVC, forced vital capacity.

Data are shown as mean±SD, median (IQR), or number (%).

P-values were obtained by chi-square test, t-test, or Mann-Whitney U-test as appropriate.

[†] Log-transformed.

[‡] Adjusted for age, sex, atopy, body mass index, and blood eosinophil.

Supplementary Table 5. Associations between serum periostin and BMI.

	β	P-value
Non-asthmatic subjects (N=230)		
Body mass index, kg/m ²		
Crude	-0.165	0.012
Adjusted [†]	-0.134	0.057
Asthma (N=206)		
Body mass index, kg/m ²		
Crude	-0.178	0.010
Adjusted [†]	-0.163	0.020

β , standardized partial regression coefficient.

[†] Adjusted for age and sex.

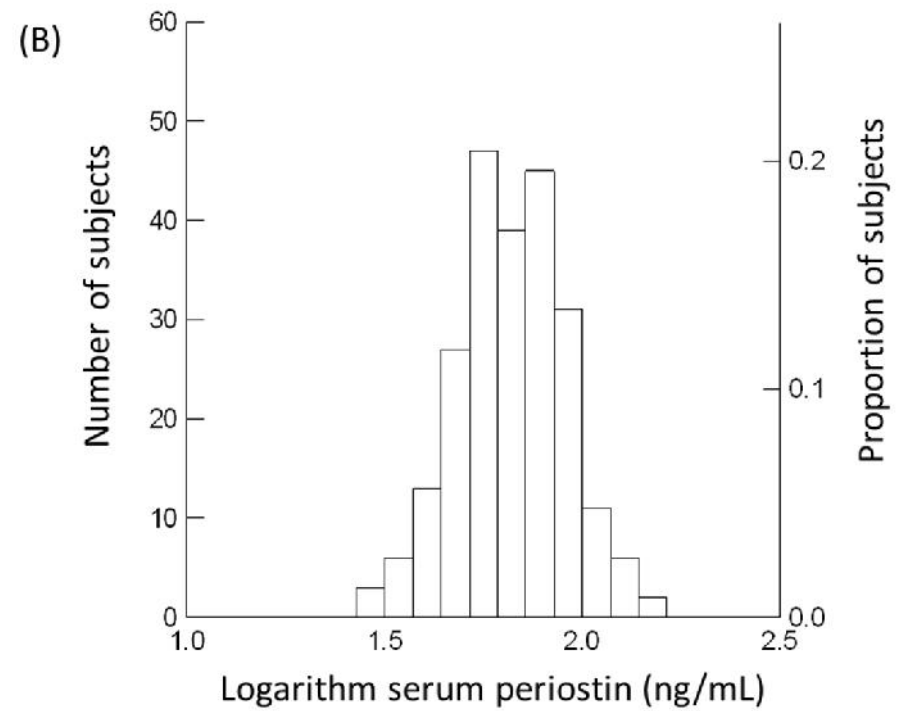
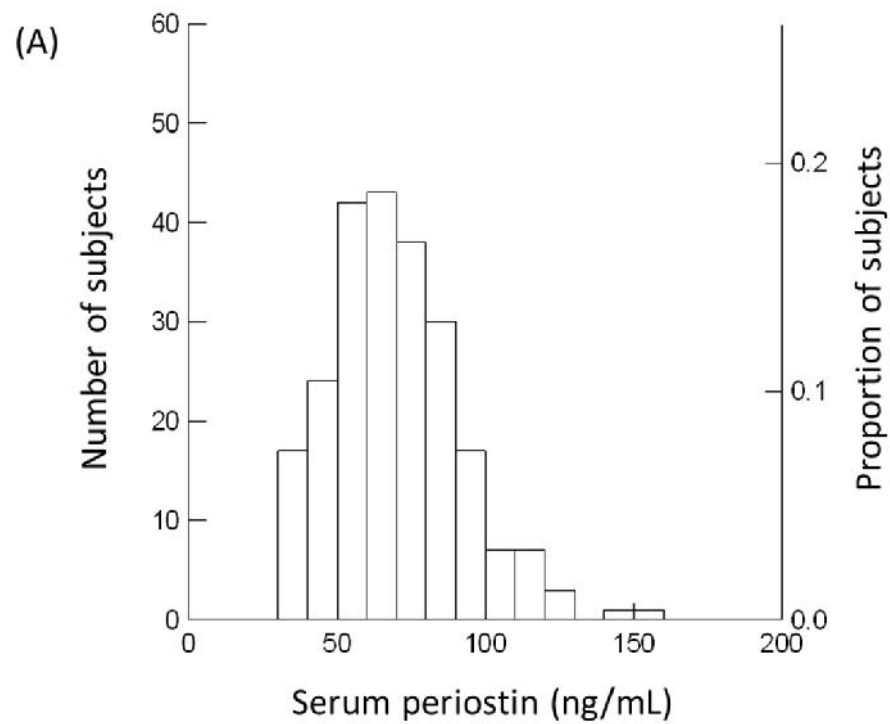
Supplementary Table 6. Clinical characteristics of asthmatic subjects with or without allergic rhinitis (AR).

	non AR (N=50)	AR (N=156)	P-value
Age, years	64.5±12.6	57.9±13.9	0.0032
Smoking status, N (%)			0.586
Current smoker	7 (14.0%)	14 (9.0%)	
Ex-smoker	23 (46.0%)	74 (47.4%)	
Never smoker	20 (40.0%)	68 (43.6%)	
Pack years	6.9 (0-24.0)	2.6 (0-13.5)	0.295
Body mass index, kg/m ²	25.8±5.4	24.7±4.9	0.169
Atopy, N (%)	29 (58.0%)	111 (71.2%)	0.083
Blood eosinophils, cells/μL [†]	182.4 (0.44)	226.0 (0.44)	0.193
Serum total IgE, IU/mL [†]	116.1 (0.87)	158.0 (0.66)	0.252
Serum periostin, ng/mL [†]	79.0 (0.14)	87.1 (0.20)	
Crude			0.173
Adjusted [‡]			0.364
FEV ₁ , % predicted	92.6±17.4	96.3±19.6	0.230
FEV ₁ /FVC, %	66.3±11.3	69.2±12.9	0.159

P-values were obtained by chi-square test, t-test, or Mann-Whitney U-test as appropriate.

[†] Log-transformed.

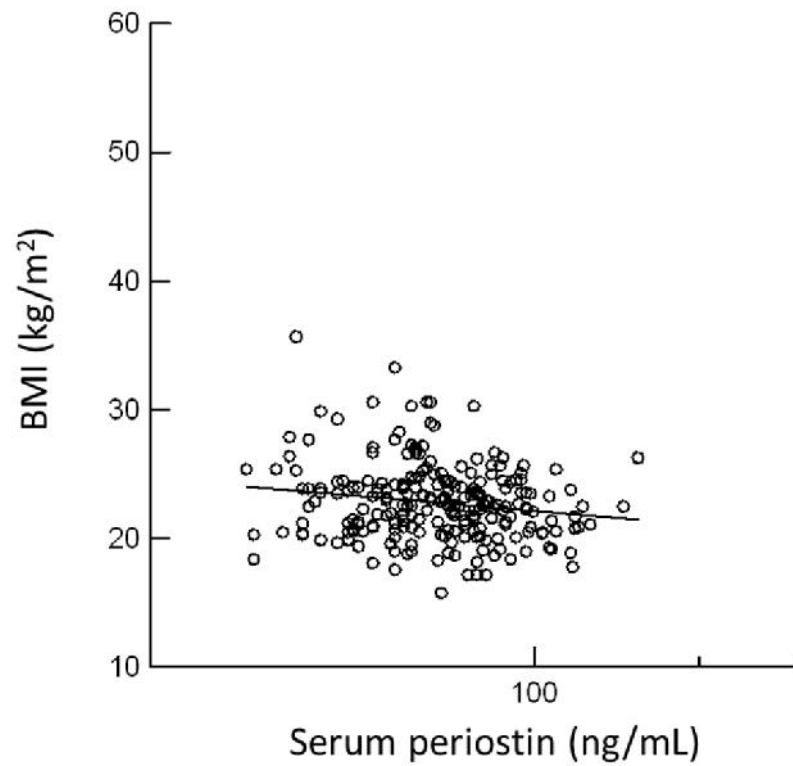
[‡] Adjusted for age, sex, atopy, BMI and blood eosinophil.



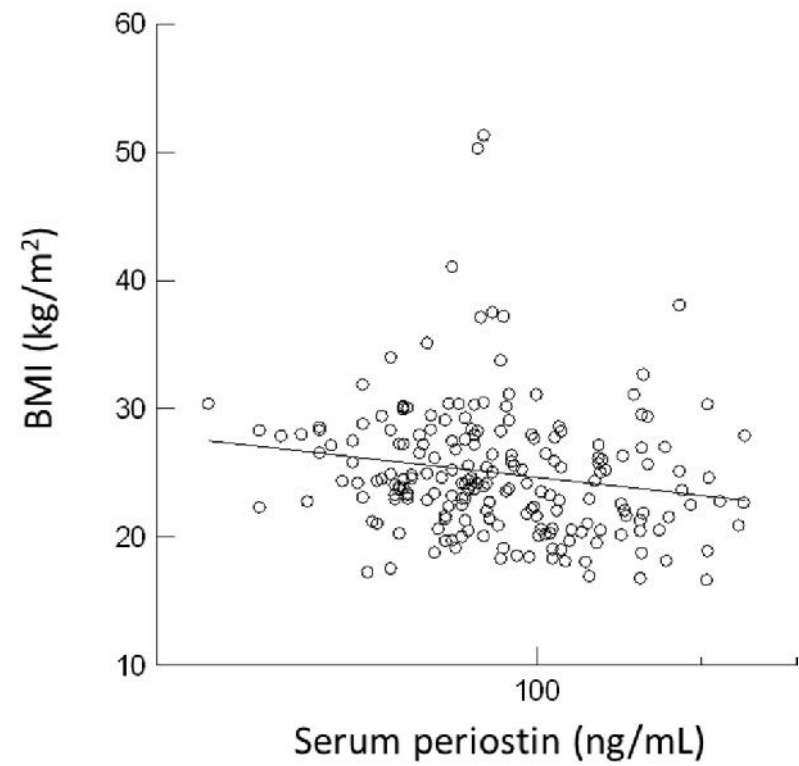
Supplementary Fig. 1.

Distribution of (A) serum periostin and (B) logarithm-transformed periostin.

(A)



(B)



Supplementary Fig. 2.

Correlation between serum periostin levels and BMI in (A) Non-asthmatic subjects (B) Asthmatic subjects

Abbreviations: BMI, body mass index.