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Serum periostin levels correlate with airway hyperresponsiveness to methacholine and mannitol in children with asthma

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INTRODUCTION

Asthma is a complex clinical syndrome characterized by variable airflow obstruction (some of which can be permanent), airway hyperresponsiveness (AHR), and cellular inflammation. It is a heterogeneous disorder, exhibiting multiple phenotypes, reflected by the many clinical aspects of the disease (1-5). Recent advances in the identification of serum biomarkers for asthma are important in its diagnosis, including determination of the asthma phenotype or endotype. Woodruff *et al.* emphasized the utility of periostin as a biomarker of T_H2-induced airway inflammation (6). Using gene expression profiling of airway epithelial brushings, they showed that the expression levels of the IL-13-responsive gene *POSTN*, which encodes periostin, could be used to divide asthmatic patients into high- and low-T_H2 phenotypes, with the former responding well to inhaled corticosteroids (ICS), but not the latter. In 2011, Lebrikizumab (a monoclonal antibody to IL-13) was reported to improve lung function, especially in patients with a high periostin levels in their serum; thus, periostin was suggested as a serum biomarker that suggests a favorable outcome in combined treatment with lebrikizumab (7). More recently, biomarker analyses showed that omalizumab significantly reduces the frequency of exacerbation in patients with high periostin levels, and that these were associated with the fraction of exhaled nitric oxide (FeNO) and eosinophil levels in the blood (8).

AHR to exogenous stimuli is a characteristic of asthma (9). It can be assessed by performing bronchial provocation tests (BPTs) using direct stimuli, such as methacholine, or indirect stimuli, including exercise, hypertonic saline, and mannitol. Periostin in the serum is one of the most promising biomarkers of T_H2-induced airway inflammation (6), eosinophilic airway inflammation (10), and the response to T_H2-targeted therapy (6). However, the

relationships between levels of periostin and other markers of inflammation remain unclear, and the interactions between periostin and AHR are not understood.

We hypothesized that periostin levels induced by IL-13 would be higher in asthmatics than in healthy children, and might correlate with AHR in asthmatic children. Therefore, we explored the relationships between periostin level and AHR to methacholine and mannitol in asthmatic children.

METHODS

Subjects

All of the subjects were recruited from outpatient clinics at Hallym University Kangdong Sacred Heart Hospital, Seoul, Korea. We systematically recruited all new asthma cases at their first visits because of suspected asthma, and all diagnoses were verified by the clinical examination, pulmonary function testing, and methacholine BPT. We included patients in whom 0.5–2 years had elapsed since diagnosis. Asthma was defined as the presence of either a $\geq 12\%$ increase in the forced expiratory volume in one second (FEV₁) after bronchodilator treatment, or if < 16.0 mg/mL inhaled methacholine induced a 20% decrease in FEV₁ (PC₂₀) (11). Asthma severity was classified following the guidelines of the Global Initiative for Asthma (GINA), using an algorithm including medication dose, FEV₁, medication adherence, and symptom levels (12). Patients were given inhaled short-acting β_2 -agonists on demand to relieve symptoms, with or without controller medications (inhaled corticosteroids, a leukotriene receptor modifier, or long-acting β_2 -agonists). The controls were healthy children matched by age and gender, who had no history of wheezing or infection during the 2 weeks before the study. Exclusion criteria included an acute exacerbation of asthma requiring systemic corticosteroids during the previous 6 months, and parenchymal lung disease evident on chest radiographs performed 4 weeks before the study. Of the healthy controls, those at any risk of atopy or subclinical eosinophilic inflammation were excluded, using an FeNO of 20 parts per billion (ppb) or greater (13). We defined atopy as the presence of at least one positive allergen-specific IgE test result (IgE ≥ 0.35 kU/L) or a finding on skin prick testing (SPT). The spirometry and mannitol challenge tests were performed by a trained technician.

Study protocol

A schema of the study design is shown in Figure 1. After a 4-week run-in period, the patients made three visits to our clinic at the same time of day. During the observation period, all patients were asked to discontinue controller medications, and were excluded if they experienced asthma exacerbations requiring recommencement of such medication. At the first of the three visits, blood samples were taken and FeNO levels were measured by a physician. Each subject was evaluated using SPT and pre- and post-bronchodilator spirometry. At the second and third visits, separated by intervals of at least 1 week, BPTs with methacholine and

inhaled mannitol were performed. Blood samples were stored at -70°C before determining the periostin levels in serum. All procedures were approved by the Medical Ethics Committee of Hallym University Kangdong Sacred Heart Hospital, Seoul, Korea, and all subjects and parents gave written informed consent.

Mannitol BPT

Dry powdered mannitol (Aridol; Pharmaxis, French's Forest, NSW, Australia) was administered according to the manufacturer's recommendations, and FEV₁ values were recorded as prescribed by current guidelines (14). The FEV₁ recorded after inhalation of a placebo capsule served as the baseline value. The challenge was completed when a $\geq 15\%$ fall in FEV₁ from baseline occurred, which was considered to be a positive response, or when the maximum cumulative dose of mannitol (635 mg) was administered. Asthmatics exhibiting a decrease in FEV₁ of at least 15% from baseline after inhalation of 635 mg or less of mannitol were enrolled in the positive-mannitol BPT group and the others were placed in the negative-mannitol BPT group.

For positive challenge results, the cumulative provocative dose causing a 15% fall in FEV₁ (the PD₁₅ dose) was calculated by log-linear interpolation of the final two data points. The responses to mannitol are expressed as PD₁₅ values.

FeNO

FeNO was measured using a portable nitric oxide analyzer (NIOX MINO[®]; Aerocrine, Solna, Sweden) that provided measurements at a 50 mL/s exhalation flow rate expressed in parts per billion (ppb) (15). Determinations made with the device were in clinically acceptable agreement with measurements provided by a stationary analyzer according to the guidelines of the American Thoracic Society. (16).

Measurement of serum biomarkers

Blood samples were obtained between 8:00 and 9:00 a.m. Periostin levels were measured by the Shino test (Kanagawa, Japan), using an enzyme-linked immunosorbent assay, as described previously (17).

Statistical analysis

The data were analyzed using SPSS ver. 21.0 (SPSS, Chicago, IL). Continuous data were expressed either as means with the standard deviation or as medians with the interquartile

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ranges, depending on the data distribution. The Mann–Whitney test was performed for continuous variables and the chi-square test for categorical variables to compare the study groups. Numerical parameters with non-normal distributions (periostin level and methacholine PC₂₀ and mannitol PD₁₅ values) were log-transformed. Correlations between the periostin level, lung function, total IgE level, eosinophil count in peripheral blood (PB), eosinophil cationic protein (ECP) levels, and FeNO values were evaluated by calculating Spearman's rho. The effects of the log-transformed periostin levels on the log-transformed methacholine PC₂₀ and mannitol PD₁₅ data were analyzed using linear regression to allow adjustment for age, gender, atopy, and PB eosinophil counts. The estimates obtained were regression slopes for log-transformed periostin levels against the log-transformed methacholine PC₂₀ and mannitol PD₁₅ values.

The overall test performance of periostin for identifying asthmatic patients was reviewed using receiver operating characteristic (ROC) curve analysis. The overall accuracy of the test was measured as the area under the ROC curve. The cut-off values for periostin were found by ROC curve analysis as the values with the highest accuracy (minimal false-negative and false-positive results). The prevalence of disease used in the analysis was estimated from the ratio of positive and negative cases in the dataset. The 95% confidence interval (CI) for test characteristics was calculated using MedCalc v.14.8.1 (MedCalc, Mariakerke, Belgium).

RESULTS

Characteristics of study subjects

A total of 87 subjects were recruited and took part in this study. The patient group comprised 58 asthmatics and the control group 29 healthy subjects. During the run-in period, 4 subjects with asthma dropped out because they failed in discontinuing controller medications due to asthma exacerbation. Eighty three subjects who finished the study were enrolled in the final analysis (54 asthmatics and 29 controls). The demographic data and pulmonary function parameters of the subjects are summarized in Table 1. There were no differences between the asthmatic and healthy children in age, gender, or body mass index (BMI). Of the 54 subjects with asthma, 14 had mild intermittent asthma, 19 had mild persistent asthma, and 21 had moderate asthma, according to the GINA guidelines. The baseline FEV₁ levels and FEV₁/forced vital capacity (FVC) ratios were significantly lower in asthmatics than in healthy controls. The bronchodilator responses were significantly greater in asthmatics. Of the 54 subjects with asthma, all had a positive methacholine BPT and 38 had a positive mannitol BPT.

Biomarkers in the PB of study subjects

Biomarker levels are shown in Table 2. The total IgE level, PB eosinophil count, ECP, and FeNO differed significantly between groups. Periostin levels were significantly higher in asthmatics

than in healthy controls (76.0 (65.0-91.8) vs. 71.0 (57.5-80.0) ng/mL, $P = 0.017$; Table 2, Figure 2). No significant correlations were found between serum periostin levels, ages and gender in the both groups. Figure 3 shows the ROC curves for using periostin levels to identify asthmatics. For discriminating asthmatic patients and healthy subjects, the ROC curve had an AUC of 0.63. For this analysis, a cutoff value of 90 ng/mL had a sensitivity of 28% (95% CI 17–42%), specificity of 97% (95% CI 82–99%), positive predictive value (PPV) of 47% (95% CI 8–90%), and negative predictive value (NPV) of 92% (95% CI 84–97%) (Table 3). This table also includes a hypothetical scenario, in which the prevalence of current asthma was 10%.

Associations between periostin levels and lung function, total IgE, PB eosinophil count, ECP, and FeNO

As shown in Table 4, periostin levels were not significantly correlated with lung function, but were significantly correlated with both PB eosinophil levels (Spearman's correlation coefficient $r = 0.331$, $P = 0.015$) and FeNO ($r = 0.384$, $P = 0.004$) (Table 4). No significant correlations were found between periostin levels and ECP or the total IgE level.

Associations between periostin levels and AHR to methacholine and mannitol

Periostin levels were significantly negatively correlated with the methacholine PC₂₀ ($n = 54$, $r = -0.286$, $P = 0.036$; Figure 4A) and mannitol PD₁₅ values ($n = 38$, $r = -0.455$, $P = 0.004$; Figure 4B) in asthmatics. After adjusting for age, gender, atopy, and PB eosinophil levels, the log(serum periostin level) was significantly associated with both the log(methacholine PC₂₀) (exhibiting a 1.225% decrease for each doubling of the biomarker level from baseline, $P = 0.045$) and log(mannitol PD₁₅) (a 1.660% decrease; $P = 0.033$) (Table 5).

DISCUSSION

Periostin levels in the serum were significantly higher in asthmatics than healthy controls, which is consistent with the data of Woodruff et al. (6), Jia et al. (18), and Lopez-Guisa et al. (19) and provides additional evidence that periostin is associated with asthma. Woodruff et al. (6) performed genome-wide profiling of airway epithelia from adult asthmatics and healthy controls, and found that periostin was differentially upregulated in epithelial tissue from adult asthmatics compared to healthy subjects and smokers. *In vitro* studies have shown that airway epithelial cells from children with allergic asthma express higher levels of periostin than do cells of children without asthma (19).

Periostin (encoded by *POSTN*) is a matricellular protein expressed by connective tissue, bone, myocardium, heart valves, and skin (20, 21). It is believed to interact with several integrins and is thought to be involved in cell proliferation and regeneration, the epithelial-

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mesenchymal transformation, fibrosis, and angiogenesis (22-24). We postulated that periostin levels would be significantly higher in children compared to adults, because of the higher bone turnover and growth in children. However, the levels in our study subjects aged 6–15 years were no higher than published values for adults (7, 18) and were not significantly associated with age. No significant correlations were found between serum periostin levels and gender in asthmatics and controls. These outcomes are similar to those of previous study (18). As few data on periostin levels in infants and children are available, such values should be investigated further in both asthmatics and healthy controls (infants and children).

Although bronchoscopy, induced sputum sampling, and measurement of exhaled gases enable the direct characterization of inflammatory pathways in asthmatic airways, the use of such modalities can be time-consuming, expensive, and invasive; in addition, the techniques might not be widely available in primary care settings. In recent years, other noninvasive biomarkers of asthma severity and airway inflammation have been described. Markers of particular interest are FeNO, PB eosinophils, and serum IgE. We measured the levels of these biomarkers in asthmatic patients and normal healthy subjects. As we excluded those at any risk of atopy or subclinical eosinophilic inflammation from the healthy controls using these biomarkers, when selecting the healthy controls, it might be inappropriate to compare these markers with periostin for discriminating asthmatic patients and healthy controls.

Traditionally, asthma is thought to result from aeroallergen-induced inflammation driven by T_H2 processes (25) and is commonly characterized by eosinophilic infiltration of the airways. Jia et al. (18) reported an association between PB and airway eosinophil counts, and periostin levels in PB. They showed that periostin levels in PB identified asthmatic patients with increased airway eosinophil numbers and might therefore be clinically useful when selecting patients for T_H2 -targeted asthma therapies. The FeNO level is correlated with the total eosinophil count in asthmatic patients (26). In the present study, periostin levels in serum were significantly correlated with both the PB eosinophil level and FeNO.

We found that periostin levels were well correlated with the BPT results measured using both methacholine- and mannitol-induced AHR. The features of bronchoconstriction induced by methacholine and mannitol differ. Mannitol challenge triggers airway smooth muscle contraction via one or more intermediate mechanisms, such as the release of mediators including leukotrienes, prostaglandins, and histamine by inflammatory cells, causing airway inflammation (27), whereas methacholine acts directly on smooth muscle receptors in airway passages, triggering constriction independent of inflammation (27). Mannitol tests show high specificity when used to identify current asthma status, compared to the methacholine challenge test, and mannitol data correlate better with the extent of airway inflammation and disease severity (28, 29).

The precise sequence of events leading to the persistence of airway inflammatory cells, changes in the airway structure, changes in smooth muscle function, and AHR in asthma patients remains poorly understood. In the asthmatic airway, periostin is a newly identified

component of the thickened basement membrane and is involved in asthma fibrosis, a characteristic feature of airway remodeling (30-32). Previous studies support the notion that the T_H2 cytokine IL-13 stimulates lung fibroblasts (31) and airway epithelial cells (6, 30) to produce periostin. Periostin was speculated in the present study to be involved in chronicity of airway inflammation and AHR in asthma subjects, although further study is required to clarify this association.

There are several limitations in the present study. First, the sample size is small. Second, we cannot discuss how our findings might be linked to poor asthma control, because we did not have a group of patients exhibiting frequent exacerbations of asthmatic symptoms. Third, although our data suggest that there are significant differences in periostin levels between healthy and asthmatic patients, the ROC curve for periostin levels had an AUC of 0.63. It seems that a determination using periostin levels in isolation has limited value for predicting asthma. Combination with other biomarkers might be useful, and therefore, further large randomized clinical trials will be necessary.

On the contrary, this study has the following merits. To the best of our knowledge, this is the first controlled observational study of the relationship between periostin levels in the serum and AHR in asthmatic children. We also assessed AHR by performing both methacholine and mannitol challenge tests.

In conclusion, we found that periostin levels in the serum were significantly higher in the asthmatic group, and were significantly correlated with methacholine- and mannitol-induced AHR. Therefore, periostin levels in PB might serve as a clinically useful biomarker in asthmatic children.

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Table 1. Characteristics of study subjects.

	Asthmatics (n=54)	Healthy controls (n=29)	P value*
Age (years)	8.7 ± 1.8	8.6 ± 1.5	0.838
BMI (kg/m ²)	18.67 ± 3.28	18.36 ± 3.39	0.373
Gender (% male)	64.8	62.1	0.680 [†]
Prior ICS use, n (%)	42 (77.8%)	NA	NA
Atopy, n (%)	44(81.5%)	NA	NA
Asthma severity			
Mild intermittent, n (%)	14 (25.9%)	NA	NA
Mild persistent, n (%)	19 (35.2%)	NA	NA
Moderate, n (%)	21 (38.9%)	NA	NA
Lung function			
FEV ₁ (pred %)	84.2 ± 11.8	102.5 ± 9.9	<0.001
FVC (pred %)	90.5 ± 10.4	96.9 ± 89.7	0.114
FEV ₁ /FVC ratio	0.822 ± 0.061	0.916 ± 0.061	<0.001
Postbronchodilatory ΔFEV ₁ (pred %)	9.2 ± 7.7	0.4 ± 3.8	<0.001
Mannitol PD ₁₅ (mg)	160.7 (90.3-382.1)	NA	NA

Methacholine PC ₂₀ (mg/mL)	2.9 ± 2.8	NA	NA
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Abbreviations: BMI = body mass index; ICS = inhaled corticosteroid; NA = not applicable; pred % = predicted %; PD₁₅ = the cumulative provocative dose causing a 15% fall in FEV₁; PC₂₀ = the provocative concentration of methacholine inducing a 20% fall in FEV₁.

Data are presented as absolute numbers, or as means ± standard deviations.

*Mann-Whitney test.

†Chi-squared test.

Table 2. Biomarkers in peripheral blood, and FeNO in study subjects.

	Asthmatics (n=54)	Healthy controls (n = 29)	P value*
Periostin (ng/mL)	76.0 (65.0-91.8)	71.0 (57.5-80.0)	0.017
Total IgE (IU/mL)	210.0 (91.8-475.0)	62.4 (29.3-85.6)	< 0.001
PB eosinophil (/mL)	475.0 (247.5- 787.5)	125.0 (95.0-175.0)	< 0.001
ECP (ng/mL)	42.75 (20.4-91.2)	6.3 (5.1-13.4)	< 0.001
FeNO (ppb)	23.0 (12.0-35.0)	11.0 (8.0-13.5)	< 0.001

Data are presented as means ± standard deviations, or medians (with interquartile ranges).

*Mann-Whitney test.

Table 3. The ROC curve analysis of the serum periostin levels for identifying asmathics

Periostin	AUC	Sensitivity,	Specificity,	PPV,	NPV,
Cut off, ng/mL	(95%CI)	% (95% CI)	% (95% CI)	% (95% CI)	% (95% CI)
90	0.63 (0.52-0.74)	28 (17 - 42)	97 (82-99)	47 (8-90)	92 (84 - 97)

AUC, area under curve; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value

Table 4. Spearman's correlation coefficients among study subjects.

	Serum periostin (ng/mL)	
	r	P-value
FEV ₁ (pred %)	0.073	0.602
FVC (pred %)	0.120	0.387
FEV ₁ /FVC ratio	-0.007	0.612
FEF ₂₅₋₇₅ (pred %)	0.137	0.324
PB eosinophils (/ml)	0.331	0.015
FeNO (ppb)	0.384	0.004
Total IgE (IU/ml)	0.277	0.119
ECP (ng/ml)	0.044	0.886

Table 5. The results of multiple linear regression modeling of changes in Methacholine PC₂₀ and Mannitol PD₁₅ in asthmatic children.

	Log Methacholine PC ₂₀			Log Mannitol PD ₁₅		
	Estimate	95% CI	P value	Estimate	95% CI	P value
Gender	.050	-.0527 to 1.606	.314	-0.035	-0.328 to 0.259	0.812
Age	-.030	-0.094 to 0.035	.358	0.018	-0.073 to 0.110	0.683
BMI	.002	-0.032 to 0.037	.894	-0.011	-.0063 to 0.040	0.659
Atopy	.295	0.026 to 0.564	.032	0.258	0.343 to 0.701	0.061
Log periostin level	-1.225	-2.423 to -0.027	.045	-1.660	-3.173 to -0.146	0.033

* P values were computed using a regression model evaluating differences of estimates (slopes) from zero.

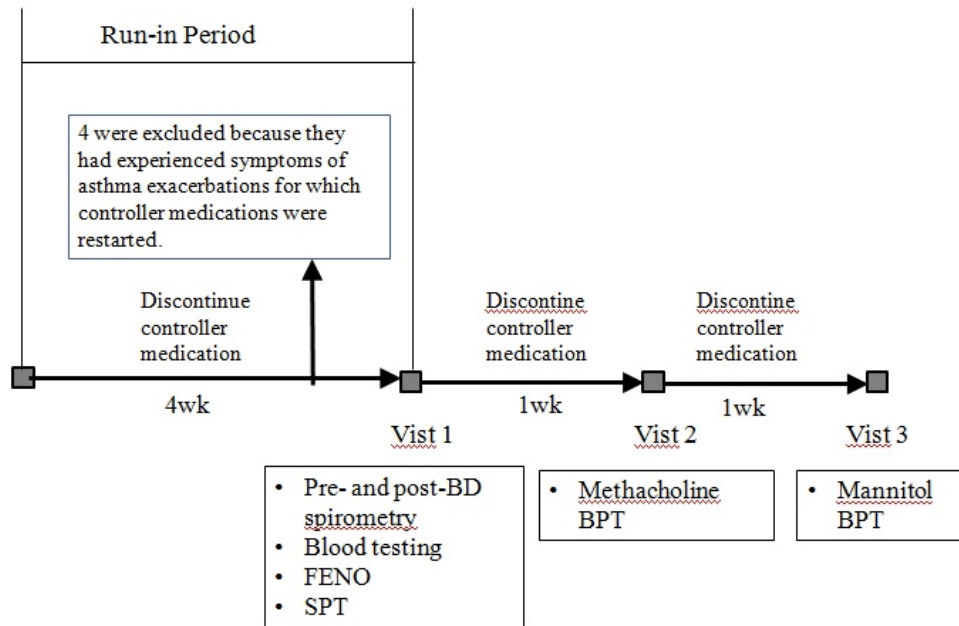


Figure 1. Study schema.

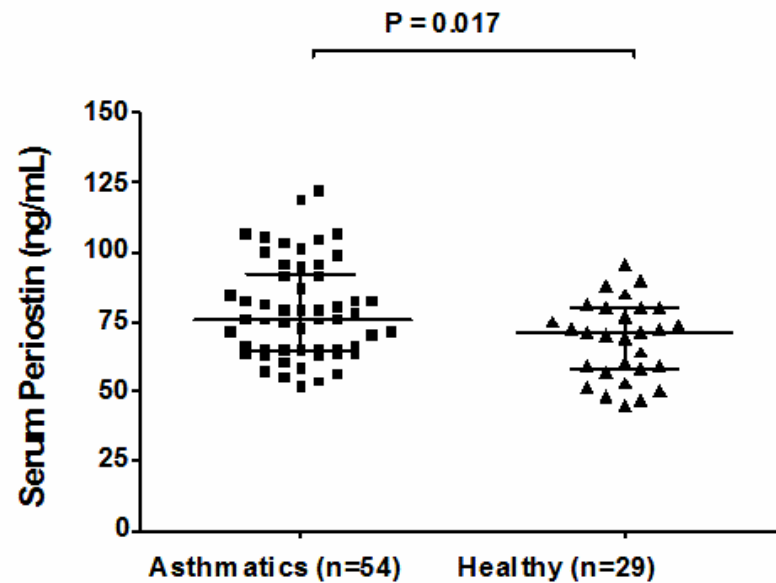


Figure 2. Asthmatics had significantly higher levels of serum periostin than did controls (76.0 (65.0-91.8) vs. 71.0 (57.5-80.0) ng/mL; $P = 0.017$). The horizontal lines in the dot plots represent median and interquartile ranges.

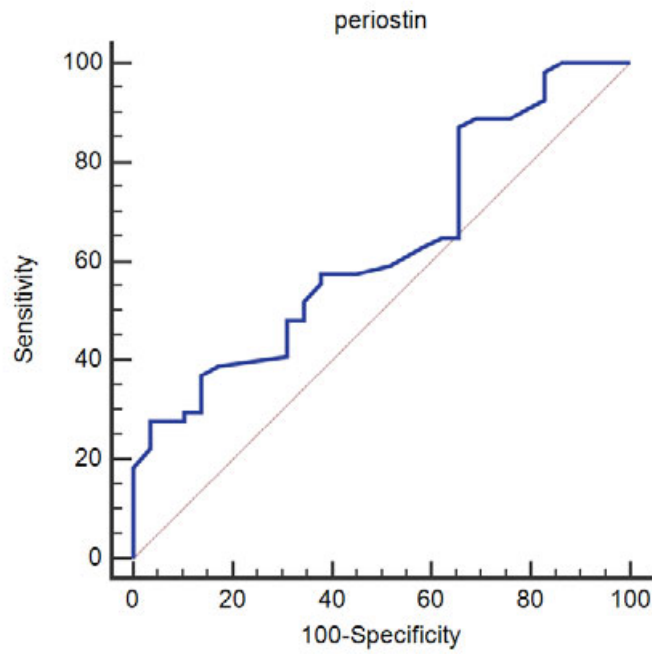
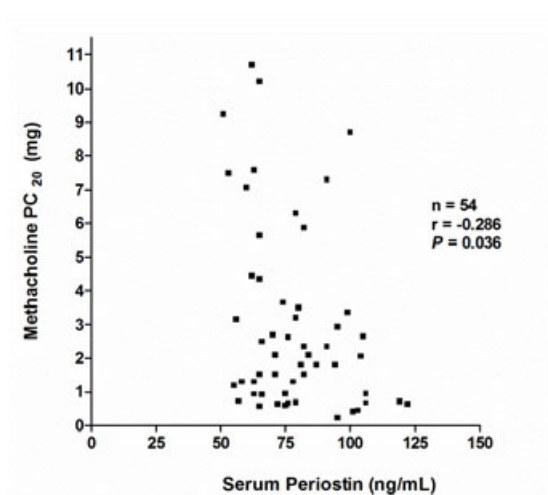


Figure 3. Receiver operating characteristic curve for periostin levels for predicting asthma.

(A)



(B)

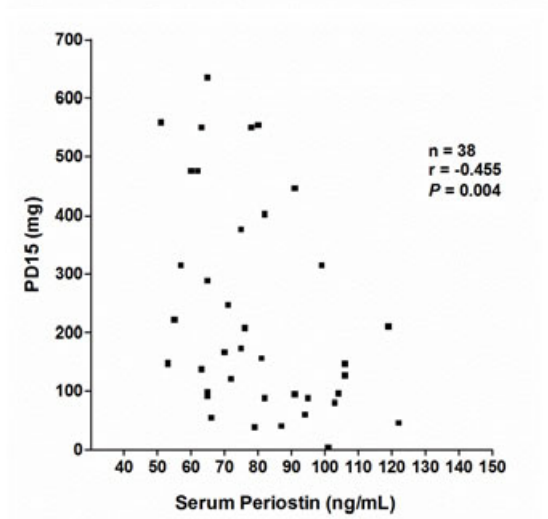


Figure 4. A: Relationship between serum periostin level and methacholine PC₂₀ in asthma subjects (n = 54). (r [Spearman's correlation coefficient] = 0.486, P = 0.002). B, Relationship between serum periostin level and mannitol PD₁₅ in asthmatic subjects (n = 38). (r = 0.486, P = 0.002).

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