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Periostin as a Biomarker for Diagnosis of Pediatric Asthma

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Running title

Periostin in pediatric asthma

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Periostin as a Biomarker for the Diagnosis of Pediatric Asthma

Pediatr Allergy Immunol

Abstract

Background:

There are some biomarkers for asthma diagnosis but they are often difficult in clinical use, particularly in pediatric cases. Periostin is an extracellular matrix protein, upregulated in response to IL-4 or IL-13. Serum periostin is expected to be used as a non-invasive biomarker for asthma diagnosis and management.

Method:

Twenty eight asthmatic children (BA) and 27 non-asthmatic children (patients with pectus excavatum etc. as control group) aged 6-16 years were included. Bronchial asthma was diagnosed according to International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire. Fractional exhaled nitric oxide (FeNO), lung function, blood eosinophil counts, total immunoglobulin E (IgE) levels, and serum periostin levels were assessed. Results were compared between BA and controls. Asthma diagnostic accuracies were calculated using receiver operating characteristics (ROC) curve analyses.

Results:

Serum periostin levels in the BA group were significantly higher than those in the control group [medians (with interquartile ranges), 134.0 (116.3–166.3) vs. 112.0 (97.0–132.0) ng/mL; $P = 0.012$]. The area under the ROC curve (AUC) for periostin, FeNO, and eosinophil counts were 0.70, 0.72, and 0.84, respectively. After exclusion of controls with pectus excavatum, AUC for periostin, forced expiratory volume in 1 second (FEV₁), and maximum mid-expiratory flow Rate (MMF) were 0.75, 0.74, and 0.80, respectively.

Conclusion:

Serum periostin levels were significantly higher in asthmatic children. ROC AUC values for periostin were equivalent to conventional biomarkers, including FeNO levels and lung function testing, indicating the utility of serum periostin levels in diagnosing asthma in children.

Key words

asthma; biomarker; children; FeNO; lung function; periostin; ROC curve

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Introduction

Periostin is an extracellular matrix protein expressed in fibroblasts or epithelial cells.

Takayama and Izuhara *et al.* found that T-helper cell 2 (Th2) cytokines induced periostin

expression in fibroblasts, which was involved in subepithelial fibrosis in asthma (1). In

fact, significant periostin expression has been noted in the bronchial epithelial cells of

children with asthma (2), and a study of severe adult asthma indicated that serum

periostin levels could be used as a biomarker of eosinophilic airway inflammation (3).

Another study reported that high serum periostin levels correlated with a decrease in the

forced expiratory volume in 1 second (FEV₁) by age (4). Lebrikizumab has been reported

to be effective in treating patients with asthma with high levels of serum periostin (5);

serum periostin levels have also proven to be useful in determining the effectiveness of

omalizumab in treating asthma (6). Thus, research suggests that periostin serves as a

powerful biomarker in the treatment of asthma.

Squamous cell carcinoma antigen (SCCA) is a protein extracted from cervical cancer (7);

its expression is increased in the airway tissues of asthmatic patients (8), suggesting that

both periostin and SCCA are related to the pathogenesis of asthma.

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Biomarkers known to be useful for diagnosis of asthma include the sputum eosinophil count, lung function measurement, airway reversibility testing, airway hyperreactivity testing, and fractional exhaled nitric oxide (FeNO) measurement (9). However, these measurements are not practical for routine use in general practice. The diagnosis and objective assessment of pediatric asthma is not easy; therefore, there is a need for a simple, reliable, and minimally invasive biomarker.

The current study compared the use of serum periostin levels against other potential biomarkers to determine its usefulness as a biomarker for diagnosis of pediatric asthma.

Methods

Patients

Children with asthma (asthmatic group) were enrolled at two specialist allergy facilities (Jikei University Daisan Hospital and Sagamihara National Hospital), and children without asthma, patients with pectus excavatum (PE) etc. as control group, were enrolled at three facilities (Jikei University Hospital, Jikei University Daisan Hospital and Sagamihara National Hospital) from June 2013 to January 2015. They were aged 6–16 years. The ethics committees of the participating institutions approved this study and written informed consent was obtained from a parent/guardian of each of the participants.

The presence or absence of asthma was determined based on responses to the International Study of Asthma and Allergies in Childhood questionnaire (ISAAC). The severity and level of asthma control were categorized in accordance with the 2014 criteria of the Global Initiative for Asthma (GINA). From the results of the questionnaire-based survey, we excluded patients with conditions that may affect serum periostin levels, including those with heart disease, kidney disease, bone disease, atopic dermatitis (AD), or malignancies; those who had suffered an asthma attack in the last 30 days; and those who were deemed to have an acute infection. At the start of this research, we did not have enough information concerning serum periostin levels in children. However, in an adult population, serum periostin levels of approximately 28 ng/mL and 8 ng/mL characterized patients with asthma and controls, respectively (the standard deviation was assumed to be 10 ng/mL). With an error of 0.05 and a power of 0.8, the total sample size required was calculated to be 17 patients in each group. However, 25 patients were finally set in each group for achieving more accurate receiver operating characteristic (ROC) curves.

Measurements and testing

Blood sampling, lung function testing, and FeNO measurement were performed on the same day, after obtaining consent. The asthmatic group also underwent an airway hyperreactivity test with methacholine. Results were compared between BA and control

group. ROC curves were plotted in both comparisons to visualize the usefulness of periostin for diagnosis of asthma.

Lung function was measured with an Autospiro (Minato, Japan). FeNO was measured with a Niox Mino device (Aerocrine, Solna, Sweden). All testing and measurements were performed by either a trained pediatrician or a laboratory technician.

Airway hypersensitivity test with methacholine

Spirometry was performed with an Autospiro (Minato, Japan) using standard techniques.

Methacholine was delivered by a nebulizer (Model 646; Devilbis; Somerset, PA).

Medications, including inhaled β_2 stimulants, steroids, leukotriene-receptor antagonists, and antihistamines, were stopped at least 24 h prior to testing. Participants then inhaled increasing doses of methacholine starting at 0.032 mg/mL and were progressively doubled up to 16 mg/mL. The provocative concentration of methacholine that caused a 20% fall in the FEV₁ (i.e., the PC₂₀) was recorded.

Measurement of systemic biomarkers

After blood was taken, serum samples were stored at -20°C and we measured the serum levels of total immunoglobulin E (IgE), house dust mite-specific IgE, periostin, and squamous cell carcinoma antigen (SCCA) 1 and 2. Serum periostin levels were measured with an enzyme-linked immunosorbent assay (ELISA) at Shino-test (Kanagawa, Japan), as previously described (4). Serum SCCA levels were measured with an ELISA, as previously described (10).

Statistical analysis

Data from this study were analyzed using Prism 5.0 (GraphPad Software). Analysis was performed using the Mann-Whitney U test for continuous variables and the Pearson chi-square test for dichotomous variables. The ROC curves were analyzed to identify suitable biomarkers for diagnosing asthma, and to provide optimal cut-off values when biomarkers were identified. The correlation coefficients between periostin and other clinical indices were assessed using Spearman's rank correlation coefficient. Data are presented as the medians (with interquartile ranges).

Results

Patients' characteristics

We enrolled 55 children aged 6–16 years, with 28 and 27 in the asthmatic and control groups, respectively. Patients' characteristics are summarized in Table 1. No significant differences were found between the asthmatic and control groups in terms of age, sex, body mass index, food allergies. However, a history of allergic rhinitis was higher in the asthmatic group than that in the control group. In the asthmatic group, 19 patients (67.9%) had mild asthma, 9 patients (32.1%) had moderate asthma, and all 28 patients were well controlled. The median threshold response to methacholine was 1.2 mg/mL. The control group included 13 children with PE, 2 with congenital biliary dilatation (surgically treated), 2 with iron-deficiency anemia, 1 with cretinism, 1 with chronic thyroiditis, 1 with orthostatic intolerance, 1 with short stature, and 6 healthy children.

Levels of biomarkers in blood, lung function, and fractional exhaled nitric oxide

The results of the blood biomarker, lung function, and FeNO testing for the asthmatic and control groups are shown in Table 2. The median serum periostin level was 134.0 (116.3–166.3) ng/mL in the asthmatic group and 112.0 (97.0–132.0) ng/mL in the control group, showing that asthmatic children had significantly higher serum periostin levels ($P = 0.012$, Fig. 1). The asthmatic group also had a significantly higher FeNO [31.5

(23.5–43.7) vs. 16.0 (10.5–28.3) parts per billion (ppb), $P = 0.005$], eosinophil count [389.7 (298.5–571.1) vs. 164.0 (87.9–263.0)/ μL , $P < 0.001$], total IgE level [551.5 (328.6–1201.8) vs. 121.0 (20.3–282.0) IU/mL, $P < 0.001$], and concentration of house dust mite (*Dermatophagoides pteronyssinus*) antigen-specific IgE levels [79.5 (38.0–100.0) vs. 6.1 (0.1–22.3) kUa/mL, $P < 0.001$]. No significant differences were observed between the asthmatic and control groups in the SCCA1 and SCCA2 levels, the predicted peak expiratory flow rate (PEFR), the percentage of FEV₁ (%FEV₁), or the predicted percentage of maximum mid-expiratory flow (%MMF). However, when we excluded the subjects with PE from control group, the asthmatic group had a significant lower lung function in %PEFR, %FEV₁, and %MMF as shown in Table 2. In addition, children with periostin levels above 50% (127–259 ng/mL) had significantly higher FeNO levels than those with periostin levels below 50% (73–125 ng/mL) [34.0 (16.7–50.8) vs 19.5 (12.3–26.9) ppb, $P = 0.021$].

Receiver operating characteristic curves to identify asthmatic children

Figures 2 and 3 show the ROC curves for diagnosing asthma by the periostin level, eosinophil count, FeNO level, % FEV₁, and %MMF. The area under the ROC curve (AUC) was 0.70 for periostin, 0.84 for eosinophil count, 0.72 for FeNO (Fig 2). The optimal periostin cut-off level was 117 ng/mL, which had a sensitivity and specificity of 75.0% and

59.3%, respectively. After exclusion of controls with PE, the AUC was 0.75 for periostin, 0.74 for % FEV₁, and 0.80 for %MMF (Fig 3).

Correlation coefficient between serum periostin levels and blood biomarker, lung function, and fractional exhaled nitric oxide levels

Periostin was slightly correlated with the eosinophil count ($r_s = 0.28$, $P = 0.036$), total IgE level ($r_s = 0.30$, $P = 0.025$), and house dust mite-specific IgE level ($r_s = 0.29$, $P = 0.035$).

Periostin was not correlated with SCCA1, SCCA2, FeNO nor with the PC₂₀ of methacholine. Periostin was not correlated either with %PEFR, % FEV₁, or %MMF after exclusion of controls with PE.

Discussion

This study has two core findings. First, periostin levels were significantly higher in the asthmatic group (even in mild asthma), allowing patients with asthma to be distinguished from controls. Second, and partly based on the above findings, periostin may be a useful biomarker for diagnosis of asthma in children who either are unsuitable or have difficulty undergoing a lung function test or in whom FeNO measurement is precluded. Each of these findings will now be discussed.

In the current study, the asthmatic group had a higher median FeNO level than did the control group, suggesting that the groups were an appropriate sample. Moreover, based on our control of potential confounders, we believe that the differences in periostin levels can reasonably be presumed to be because of the differences in the airway pathology in asthma. Periostin is an extracellular matrix protein, the expression of which has been noted in the connective, bone, myocardial, and skin tissue (11) (12); elevated serum periostin levels have also been associated with AD (13). We excluded patients with AD, thereby removing the effect of this confounder. Periostin has also been identified in osteoblasts (14), and is involved in osteogenesis as well as, presumably, bone turnover (15). Given that osteogenic activity peaks in early childhood and early adolescence (16), age is another confounder. Indeed, participants in the current study had periostin levels that were clearly higher than that reported in adult populations (3). Nonetheless, the age distributions were comparable between the asthmatic [10.6 (9.0–14.0) years] and control group [11.1 (10.1–13.3) years]. The levels of serum periostin were inversely correlated according to the age in this study. However, there was no significant difference in correlation coefficient in asthmatic ($r_s = -0.085$, $P = 0.665$) and control group ($r_s = -0.091$, $P = 0.653$) in this age group.

The asthmatic group in the current study had higher periostin levels than previously reported by Song et al. (17) in a pediatric asthma population. However, this discrepancy could be due to the different mean ages of the study participants (10.6 years vs 8.7 years). The definition of normal serum periostin levels in each age group will be necessary for future practical applications. Besides periostin, both SCCA1 and SCCA2 are known to be powerful biomarkers of asthma that are expressed in airway epithelial cells following stimulation by interleukin IL-4 or IL-13 (8). In fact, elevated levels of SCCA1 and SCCA2 have been noted during asthma attacks (18). The current study also measured levels of SCCA1 and SCCA2 but no significant differences were observed, possibly because the children in this study had stable asthma symptoms. Thus, periostin, in combination with other markers, such as eosinophil count, was able to distinguish between children with stable asthma from children without asthma after controlling for key confounders. Moreover, children with periostin levels above 50% had significantly higher FeNO levels than those with periostin levels below 50% ($P = 0.021$). The current results therefore suggest that this finding was specific to airway pathology in asthma.

Our results suggest that serum periostin may have a role as a specific biomarker for asthma diagnosis. The Th2 immune response is closely related to the pathology of asthma, with stimulation by Th2 cytokines (e.g., IL-4 or IL-13) known to increase the expression of periostin by fibroblasts (1). In addition, the airway epithelial cells of

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patients with asthma show increased periostin expression compared with that of patients without asthma (2) (19) (20). These findings corroborate the fundamental contention that periostin may serve as a biomarker of early-onset allergic asthma (i.e., Th2-mediated allergic asthma) (21).

We also compared the use of periostin levels as a means of diagnosing pediatric asthma by performing AUC calculations. Notably, ROC AUC values for periostin were comparable with those for FeNO (Fig. 2; AUCs of 0.70 and 0.72 for periostin and FeNO, respectively); this indicates that periostin is comparable with FeNO in its usefulness as a biomarker for diagnosis of pediatric asthma. This trend was quite similar even after exclusion of controls with PE (AUCs of 0.74, 0.79 and 0.79 for periostin, FeNO and eosinophil counts respectively). The AUC for the peripheral eosinophil count (0.84) was higher than that of either periostin or FeNO. However, the peripheral eosinophil count cannot be considered an asthma-specific diagnostic marker (22) because eosinophil counts increase in a host of other conditions, including parasitic infection, allergic rhinitis, and AD (23). If we were to measure the serum periostin levels routinely and combine the data with the eosinophil, FeNO measurements, and lung function test, the diagnostic value would probably increase further.

In the control group, the children with PE had a significant lower %PEFR

[92.8(68.6–101.0) vs. 107.5 (85.8–119.8) %, $P=0.03$], %FEV₁ [86.1 (75.6–90.7) vs. 94.4

(87.7–101.8) IU/mL, $P=0.012$], and %MMF [68.2 (63.7–95.1) vs. 102.0 (90.3–123.0)

IU/mL, $P=0.012$] than children without PE, suggesting that PE could have affected the

respiratory function. A study reported that PE can affected the respiratory function (24).

Therefore, we excluded the PE group to analyze ROC curves for a comparison between

periostin levels and lung function. ROC AUC values for periostin were comparable with

those for %FEV₁ (0.75 vs. 0.74; Fig.3).

The current study has two main limitations. The first limitation is that many children in

the asthmatic group also had allergic rhinitis (25), which may be a confounding factor.

However, a literature search revealed that no previous studies have reported a

relationship between allergic rhinitis and serum periostin levels. In fact, most

participants in this study had comorbid allergic rhinitis; therefore, strict exclusion of

potential participants with allergic rhinitis may have precluded patient enrollment.

However, to clarify this issue, the role of allergic rhinitis certainly needs to be studied

further. The second limitation is that this study did not examine patients with severe

asthma. In the future, we aim to study the relationship between periostin and asthma

severity.

Conclusions

In the current study, patients with asthma had significantly higher serum periostin levels than control patients. We found that periostin may be a useful biomarker for diagnosis of asthma in children who are unsuitable for lung function testing or FeNO measurement. However, further study is required to assess the correlation between periostin levels and asthma severity.

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Figure legends

Figure 1.

Serum periostin levels in BA was significantly higher than in Control

[medians (with interquartile ranges): 134.0 (116.3–166.3) vs 112.0 (97.0–132.0) ng/mL].

BA, asthmatic children; Control, non-asthmatic children.

Figure 2.

ROC curve analysis of serum periostin, FeNO and blood eosinophils.

AUC, area under curve; ROC, receiver operating characteristic.

Figure 3.

ROC curve analysis of serum periostin, %MMF and %FEV₁ without pectus excavatum

AUC, area under curve; ROC, receiver operating characteristic.

Table 1 Patients' characteristics

	BA	Control	P -value
	(n = 28)	(n = 27)	
Age (years)	10.6 (9.0–14.0)	11.1 (10.1–13.3)	0.923 ¹⁾
Male sex, n (%)	17 (60.7)	13 (48.1)	0.350 ²⁾
BMI (kg/m²)	18.3 (16.7–19.5)	17.3 (15.8–18.8)	0.103 ¹⁾
Food allergy, n (%)	3 (10.7)	1 (3.7)	0.317 ²⁾
Allergic rhinitis, n (%)	25 (89.3)	3 (11.1)	<0.001 ²⁾
ICS use, n (%)	20 (71.4)	NA	NA
Asthma severity			
Mild, n (%)	19 (67.9)	NA	NA
Moderate, n (%)	9 (32.1)	NA	NA
Asthma control level			
Well controlled, n (%)	28 (100)	NA	NA
PC₂₀ of methacholine (mg/mL)	1.2 (0.6–3.3)	ND	NA

BA, asthmatic children; Control, non-asthmatic children; BMI, body mass index; SD, standard deviations; NA, not applicable; ND, not done; ICS, inhaled corticosteroid; PC₂₀, the provocative concentration of methacholine inducing a 20% fall in FEV₁

1) Mann-Whitney *U* test

2) Chi-square test

Each data are presented as absolute numbers or medians (with interquartile ranges).

Table 2 Biomarkers in blood, Lung function and FeNO

	BA (n = 28)	Control (n = 27)	P -value
Blood eosinophils (/mL)	389.7 (298.5–571.1)	164.0 (87.9–263.0) 180.1 (90.8–306.9)*	<0.001 0.002
Total IgE (IU/mL)	551.5 (328.6–1201.8)	121.0 (20.3–282.0) 101.0(20.4–286.5)*	<0.001 <0.001
House dust mite-specific IgE (kUa/mL)	79.5 (38.0–100.0)	6.1 (0.1–22.3) 8.7(0.1–25.2)*	<0.001 <0.001
Serum periostin (ng/mL)	134.0 (116.3–166.3)	112.0 (97.0–132.0) 108.0(95.5–124.8)*	0.012 0.009
FeNO (ppb)	31.5 (23.5–43.7)	16.0 (10.5–28.3) 13.0(9.0–18.0)*	0.005 0.002
SCCA1 (ng/mL)	0.8 (0.6–1.1)	0.9 (0.7–1.3) 0.8(0.5–1.0)*	0.618 0.445
SCCA2 (ng/mL)	0.5 (0.4–0.8)	0.6 (0.5–0.9) 0.6(0.5–0.7)*	0.244 0.626
Lung function			
PEFR, pred %	85.9(79.0–92.7)	94.4(82.5–114.3) 107.5(85.8–101.0)*	0.207 0.018
FEV₁, pred %	86.0(79.0–92.7)	89.4(85.7–96.4) 94.4(87.7–101.8)*	0.254 0.012
MMF, pred %	78.8(69.8–86.8)	95.1(68.1–118.1) 102.0(90.3–123.0)*	0.068 0.001

BA, asthmatic children; Control, non-asthmatic children; pred %, predicted %

*Control group without pectus excavatum

Each data represented as medians (with interquartile ranges).

Analysis was performed using the Mann-Whitney U test.

Table 3 Spearman's correlation coefficients between periostin and other clinical indices

	Serum periostin (ng/mL)	
	r_s	P -value
Blood eosinophils (μ L)	0.28	0.036
Serum IgE (IU/mL)	0.30	0.025
House dust mite-specific IgE (kUa/mL)	0.29	0.035
FeNO (ppb)	0.25	0.081
SCCA1 (ng/mL)	0.01	0.920
SCCA2 (ng/mL)	-0.03	0.837
PC ₂₀ of methacholine	-0.12	0.549
Lung function		
PEFR, pred %	-0.22	0.260*
FEV ₁ , pred %	-0.21	0.477*
MMF, pred %	-0.28	0.228*

PC₂₀, the provocative concentration of methacholine inducing a 20% fall in FEV₁; pred %,

predicted

*Spearman's correlation coefficients in asthmatic children and non-asthmatic children

without pectus excavatum

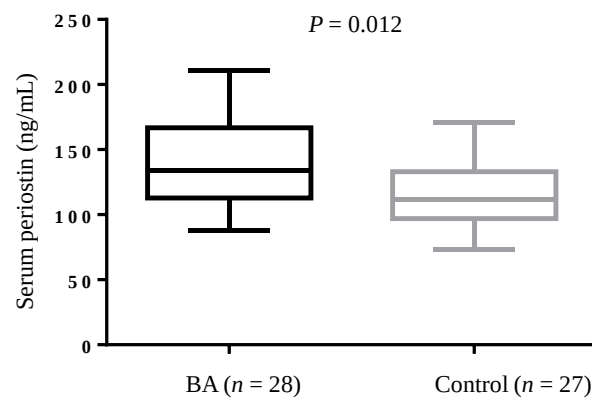


Figure 1

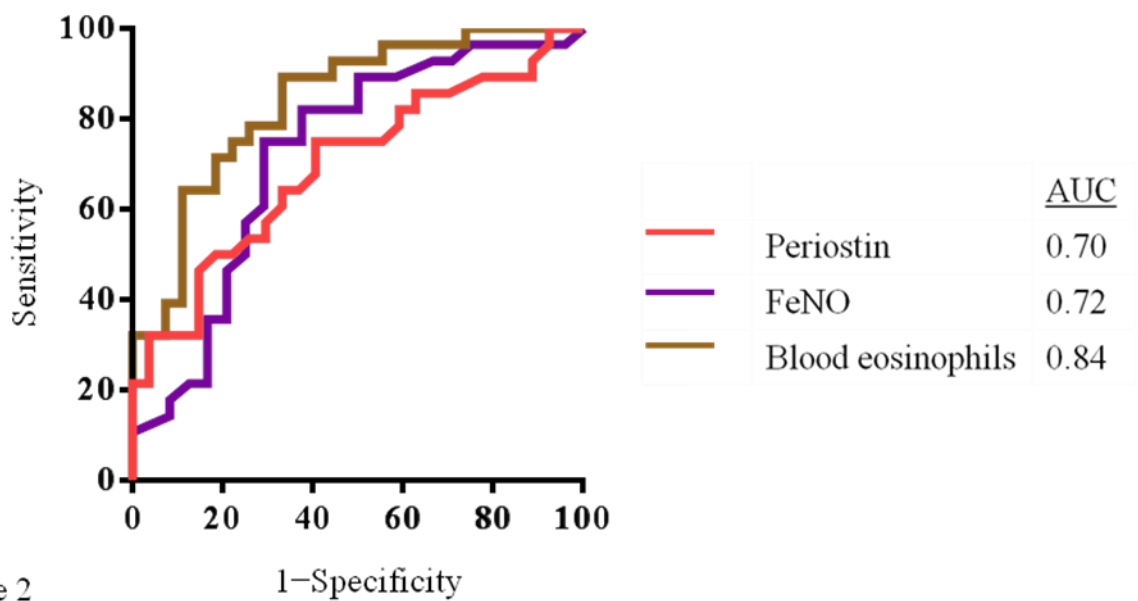


Figure 2

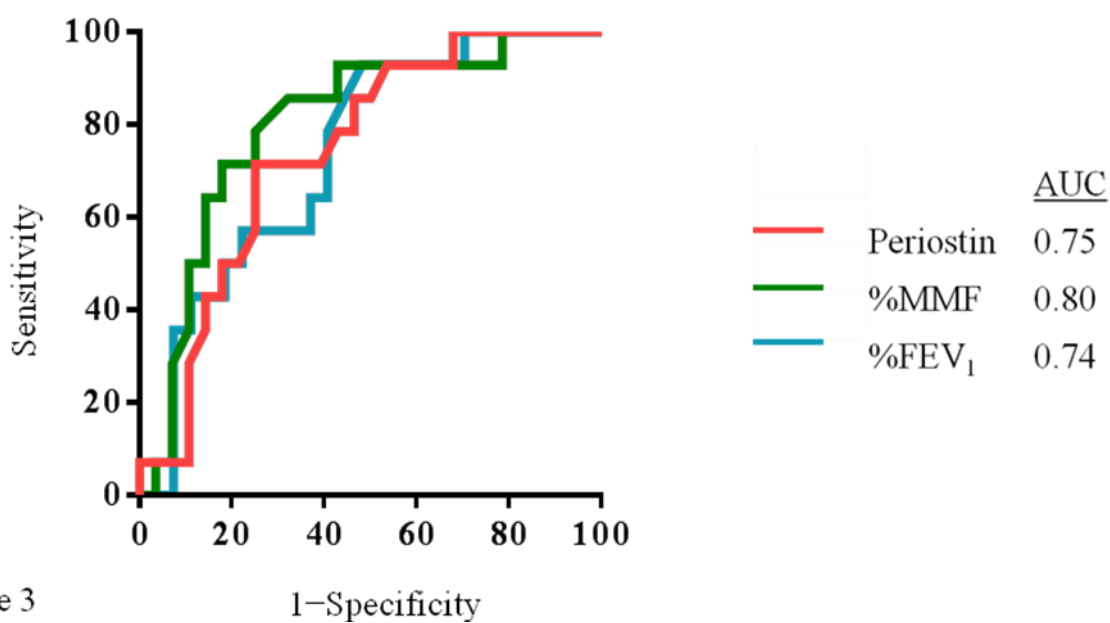


Figure 3