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Review article

Utility of serum periostin in combination with exhaled nitric oxide in the management of asthma

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Abbreviations:
FeNO, fraction of exhaled nitric oxide;
FEV_1, forced expiratory volume in 1 s;
ICS, inhaled corticosteroid;
IgE, immunoglobulin E; IL, interleukin;
iNOS, inducible NO synthase; KiHAC, Kinki Hokuriku Airway disease Conference;
TGF, transforming growth factor;
TNF, tumor necrosis factor

ABSTRACT

Type-2/eosinophilic inflammation plays a pivotal role in asthma. The identification of severe type-2/eosinophilic asthma is important for improving the management of patients with asthma. Therefore, efforts to develop non-invasive biomarkers for type-2/eosinophilic airway inflammation have been made during this decade. Currently, fraction of exhaled nitric oxide (FeNO) and serum periostin levels are considered markers of type-2/eosinophilic inflammation in asthma. However, a single-marker approach has limited the ability to diagnose severe type-2/eosinophilic asthma accurately and predict disease outcomes precisely. The present article reviews the utility of FeNO and serum periostin levels in a single-marker approach and in a multiple-marker approach in identifying patients with severe type-2/eosinophilic asthma. Furthermore, based on a sub-analysis of the Kinki Hokuriku Airway disease Conference (KiHAC), geno-endo-phenotypes of patients were stratified into four groups according to the FeNO and serum periostin levels.

Introduction

Asthma has recently been recognized as an umbrella term that encompasses various phenotypes and endotypes rather than a single disease.[1,2] Despite the diversity of endotypes and inflammatory patterns,[3] type-2/eosinophilic inflammation remains a key driver in nearly half of all patients with asthma[4] and has been demonstrated in airway epithelial cells isolated from patients with mild-to-moderate asthma.[5] Therefore, efforts to develop non-invasive biomarkers for type-2/eosinophilic airway inflammation have been made during this decade. Currently, fraction of exhaled nitric oxide (FeNO) and serum periostin levels are considered biomarkers of type-2/eosinophilic inflammation. In the present review article, the strength and weakness of FeNO and serum periostin levels as markers of type-2 inflammation are briefly summarized, which may facilitate improved interpretation of markers in the management of asthma. Studies that compared the utility of two markers to identify severe type-2/eosinophilic airway inflammation or to diagnose pediatric asthma are also reviewed. A single-marker approach may be insufficient to cover the whole range of asthma management, from disease diagnosis to prediction of disease prognosis and response to treatments, even when limited to the prediction of eosinophilic airway inflammation.[6] However, evidence regarding the use of a multiple-marker approach to identify severe type-2/eosinophilic asthma is scarce.[7,8] Herein, the potential utility of a composite marker of FeNO and serum periostin levels is presented based on a sub-analysis of the Kinki Hokuriku Airway disease Conference (KiHAC). Geno-endo-phenotypes with

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either high FeNO levels only or serum perisotin levels only are also described.

FeNO

Currently, FeNO is commonly used in the clinical settings of asthma, and the measurement of FeNO at 50 mL/s of expiratory flow using NIOX VERO® and NObreath® is generally accepted by health insurance systems, including in Japan.[9] The utility of this marker in the management of asthma has been well-established and reviewed elsewhere.[10–12] In brief, NO is predominantly produced by inducible NO synthase (iNOS), which is upregulated in airway epithelial cells, macrophages, and other inflammatory cells in response to the type-2 inflammatory milieu in asthma. Elevated FeNO levels reflect airway eosinophilic inflammation and aid the diagnosis of type-2/eosinophilic asthma in symptomatic patients with cough, wheezes, and dyspnea.[11–13] Elevated FeNO levels predict good responses to inhaled corticosteroid (ICS) treatment, particularly in ICS-naïve patients with asthma.[14,15] Basically, iNOS and FeNO levels are steroid-sensitive, and elevated FeNO levels in patients treated with ICS may indicate poor adherence to ICS.[12,14,16,17] On the other hand, elevated iNOS and FeNO may indicate ICS insensitivity or severe type-2/eosinophilic asthma,[12,18] which reflects a phenotype at an increased risk of future exacerbations.[19,20] Elevated FeNO levels also reflect oxidative/nitrative stress in the airways, which drives fibrosis progression[21] and may represent a marker of excess decline in pulmonary function when sufficiently elevated.[22,23] Thus, FeNO alone may identify severe type-2 predominant asthma in real-world settings. However, there may be a patient group, as discussed later, with high FeNO levels that are asymptomatic and stable for prolonged periods without demonstrating excess decline in pulmonary function. The mechanisms underlying the non-specific raise in FeNO levels remain unknown but may be augmented by several factors other than eosinophilic airway inflammation, such as height and male gender (Table 1). Constitutive NOS, of which sources are steroid insensitive, may also be involved.[24]

Serum periostin

Serum periostin is considered another promising biomarker of type-2/eosinophilic inflammation. Periostin expression is increased by stimulation with interleukin (IL)-4, IL-13, and transforming growth factor β mainly in airway fibroblasts and epithelial cells.[25–27] The utility of serum periostin in asthma management is also reviewed elsewhere.[27–31] Periostin, a matricellular protein, is a downstream product of the type-2 pathway; promotes eosinophil adhesion and recruitment to the airways[32]; and activates functions of eosinophils, including O_2^- generation.[33] Thus, high serum periostin levels are considered a marker of type-2/eosinophilic asthma and airway remodeling that results in an accelerated decline in pulmonary function.[34] Similar to FeNO,[35] high serum periostin levels are often accompanied by eosinophilic chronic rhinosinusitis-like conditions[29,36] and may predict treatment failure while tapering ICS doses[37] and good responses to biologics against type-2 pathway in patients with asthma.[38,39] In contrast with FeNO, serum periostin levels are stable with a small coefficient of variation[40,41] and may have a feature of ICS insensitivity.[29,42] These similar but different characteristics/modifiers indicate that high serum periostin levels may imply a more static disease process, while FeNO levels reflect more dynamic disease activity in patients with type-2/eosinophilic asthma on ICS treatment.[29] Although the precise mechanisms are unknown, elevated serum periostin levels are less frequently observed in obese patients with asthma,[43] which is also reported in a recent epidemiological study on serum periostin levels.[44] Possibly reflecting its fibrosis-prone nature,[45] serum periostin levels are elevated in fibrotic diseases, such as idiopathic interstitial pneumonia[46] and scleroderma[47] (Table 1).

Table 1
Characteristics of FeNO and serum periostin.

	FeNO	Serum periostin
Relevant cytokines	IL-4,[60,61] IL-13[60,62] IL-1β,[63] TNF-α[63]	IL-4,[25–27] IL-13[25–27] TGF-β[26,27]
Modifiers	Height ↑[64–66] Male gender ↑[66,67] Nitrate-rich diet ↑[68] Airway viral infection ↑[69] Current smoking ↓[70] Spirometric manoeuvers ↓[71] Atopic predisposition ↑[64,65,67] Allergic rhinitis ↑[65,72]	Idiopathic pulmonary fibrosis ↑[46] Scleroderma ↑[47] Bone marrow fibrosis ↑[73] Proliferative diabetic retinopathy ↑[74] Non-alcoholic fatty liver disease ↑[75] IgG4-related diseases ↑[76] Atopic dermatitis ↑[77]
Responsiveness to ICS	++[29]	+[29]
Pulmonary function decline	+[23] (when high enough)	+[34]

Comparisons between FeNO and serum periostin in the prediction of airway eosinophilia and diagnosis of pediatric asthma

Efforts to identify the best single marker with sufficient sensitivity and specificity to predict airway eosinophilia is clinically important. Although direct comparisons between FeNO levels and serum periostin levels are rarely reported (Table 2), serum periostin levels have been found to be the best predictor of airway eosinophilia among FeNO, blood eosinophil counts, serum IgE, and serum periostin in adult patients with severe asthma who remained symptomatic despite receiving high doses of ICS treatment (BOBCAT study) (n = 67; 32 males; mean age, 46 years; FEV_1, 60%; daily ICS doses >1000 μg fluticasone propionate equivalent; Asthma Control Questionnaire score, 2.7).[41] These results were not observed in another study of patients with mild-to-moderate asthma (n = 110; 54 males; mean age, 49 years; FEV_1, 100%; daily ICS doses, 500 μg fluticasone propionate equivalent).[48] However, the potential mechanisms underlying this discrepancy may be attributable to differences in periostin assay systems and disease severity among studied patients.[49] A recent study of Japanese pediatric patients with asthma reported a similar predictability of serum periostin and FeNO in distinguishing children with asthma from controls.[50] Thus, results from a single-marker approach may often depend on patient characteristics and the periostin assay kits used. Thus, a multiple-marker approach is expected to improve the accuracy in predicting severe type-2/eosinophilic asthma.

Combination of FeNO and serum periostin in the management of severe asthma

In several diseases, such as pancreatic adenocarcinoma,[51] Alzheimer's disease,[52] and severe graft-versus-host disease,[53] the superiority of a multiple-marker approach in terms of diagnostic accuracy over a single-marker approach has been reported. In mild-to-severe asthma, combinations of FeNO levels, blood eosinophil counts, and serum total IgE levels demonstrated no greater utility in predicting airway eosinophilia in asthma than single markers.[54] However, no studies of a composite marker of FeNO and serum periostin in predicting severe eosinophilic asthma have been reported.

In a sub-analysis of the Kinki Hokuriku Airway disease Conference (KiHAC) study, the utility of a composite marker of high FeNO and high serum periostin levels were examined. FeNO levels at a constant exhalation flow rate of 50 mL/s were measured using a

Table 2
Comparison between FeNO and serum periostin to predict airway eosinophilia and diagnosis of pediatric asthma.

Authors, published year	Measurement	AUC	Cutoff value	Sensitivity (%)	Specificity (%)	Target
Jia G, 2012[41]	Serum periostin	0.84	25 ng/mL	57	85	Sputum eosinophils ≥3% or total biopsy area ≥22 eosinophils/mm^2 in severe asthma
	FeNO	0.79	35 ppb	40	92	
	Blood eosinophil counts	0.71	–	–	–	
	Serum total IgE	0.62	–	–	–	
Wagener AH, 2015[48]	Serum periostin	0.55	26 ng/mL	54	57	Sputum eosinophils ≥3% in mild to moderate asthma
	FeNO	0.78	42 ppb	63	92	
	Blood eosinophil counts	0.89	270/μL	78	91	
Inoue T, 2016[50]	Serum periostin	0.70	117 ng/mL	75	59	Pediatric asthma
	FeNO	0.72	–	–	–	
	Blood eosinophil counts	0.84	–	–	–	

–, no description.

chemiluminescence analyzer (NOA 280, Sievers, Boulder, CO, USA), according to the American Thoracic Society (ATS) guidelines.[12] Serum periostin levels were measured using enzyme-linked immunosorbent assay at Shino-Test (Kanagawa, Japan). For FeNO levels, 25 ppb was used as a cutoff value because the ATS guideline recommends the consideration of 25 ppb as a cutoff value for cautious interpretation and monitoring of FeNO levels in patients on ICS treatment.[12] For serum periostin levels, 95 ng/mL was used as a cutoff value because this value had high specificity (0.985) to differentiate between patients with asthma on long-term ICS treatment and healthy subjects.[34] Because periostin expression is upregulated with the stimulation of IL-4 and IL-13[25–27] and high serum periostin levels strongly reflect airway eosinophilic inflammation,[41] it would be appropriate to consider 95 ng/mL as strictly reflecting the type 2 predominant condition when measured by the current assay system (Shino-Test, Kanagawa, Japan). A total of 121 patients receiving ICS treatment (88 females; mean age, 59 years; Asthma Control Test® score, 23 points; daily ICS doses, 525 μg equivalent to fluticasone propionate; patients with history of more than 10 pack-years were excluded) were stratified into four groups according to FeNO levels (cutoff value, 25 ppb) and serum periostin levels (cutoff value, 95 ng/mL). For the convenience of understanding, patients with low FeNO and low serum periostin levels were categorized as group A (n = 39); high FeNO and low serum periostin levels as group B (n = 34); low FeNO and high serum periostin levels as group C (n = 25); and high FeNO and high serum periostin levels as group D (n = 23) (Fig. 1).

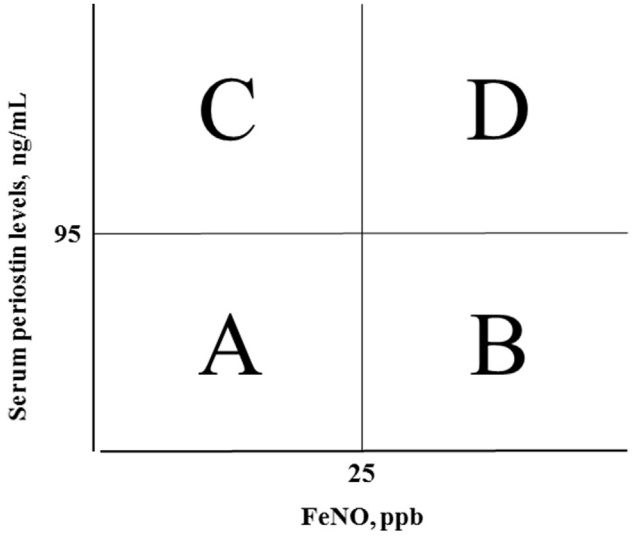

Fig. 1. Stratification of patients into four groups according to FeNO (<25 ppb, low; ≥25 ppb, high) and serum periostin levels (<95 ng/mL, low; ≥95 ng/mL, high).

To focus on the role of serum periostin in high FeNO levels (≥25 ppb), the clinical aspects of groups B and D were first compared in our previous study.[55] Patients in group D (n = 23) received more intensive treatment, had a history of asthma admission, and a decline in FEV_1 of ≥30 mL per year more frequently than those in group B (Table 3). Adherence to medications was not different between groups B and D (P = 0.56). Despite receiving intensive treatment, patients in group D had frequent asthma exacerbations that required systemic corticosteroid treatment over 2 years following enrollment (Fig. 2) and had an odds ratio of approximately 3 compared with the patients in groups A, B, and C (n = 97, one patient in group C was lost to follow-up), even after adjustment for airflow limitation (FEV_1 < 80% of predicted) and an episode of asthma exacerbation in the past 6 months. To examine if this endo-phenotype of severe type-2 inflammation was genetically associated, we examined the frequency of the GG genotype of *IL4RA* rs8832. This variant was identified in a pharmacogenetics study of pitrakinra, an inhibitor of IL-4 receptor α that is a common sub-chain for both IL-4 and IL-13 signaling, as a genetic marker of good responses to pitrakinra.[56] As expected, patients in group D had a higher frequency of the GG genotype of *IL4RA* rs8832 (35%) than the remaining patients (15%) in groups A, B, and C (Fig. 3a). Thus, high levels of both FeNO and serum periostin may identify patients with severe type-2/eosinophilic inflammation, potentially activated *via* IL-4 receptor α.

Next, geno-endo-phenotypes of patients with high FeNO levels only, high serum periostin levels only, or low levels of both are addressed (Table 3). The GG genotype of *POSTN* rs3829365 that was associated with elevated serum periostin levels[34] was the least frequent in group A (low levels of both FeNO and periostin; Fig. 3b), which was characterized by low blood eosinophil counts. A lack of elevation in type-2/eosinophilic markers may indicate genetically different backgrounds in certain patients with asthma. The frequencies of the GG genotype of *POSTN* rs3829365 were similar in groups B (high FeNO levels only) and C (high periostin levels only). The mechanism underlying the lower serum periostin levels in group B than in group C despite a similar frequency of the GG genotype of *POSTN* rs3829365 in the two groups remains unknown. Larger studies on the association between serum periostin levels and genetic background including *POSTN* and *IL4RA* would be required. Patients in group B had a significantly lower frequency of history of admission due to asthma (Fig. 3c) and were taller (Fig. 3d) than those in group C, while group C was characterized by the

Table 3
Patient characteristics in a sub-analysis of KiHAC study.

Group	Low FeNO/low periostin	High FeNO/low periostin	Low FeNO/high periostin	High FeNO/high periostin	P value*	P value**
	A (n = 39)	B (n = 34)	C (n = 25)	D (n = 23)		
Sex (F/M)	33/6	19/15	19/6	17/6	0.05	0.26
Age at enrollment, years	56 ± 14	59 ± 12	63 ± 13	60 ± 12	0.23	0.90
Age at asthma onset, years	41 ± 16	42 ± 18	35 ± 19	42 ± 16	0.46	0.99
Height, cm	157 ± 9	161 ± 8	156 ± 8	160 ± 7	0.01	0.52
Body mass index, kg/m^2	23.6 ± 3.6	23.7 ± 2.7	22.5 ± 3.1	22.1 ± 2.2	0.24	0.02
Smoking history, ex (%)	23	24	20	26	0.97	0.83
Disease duration, years	15 ± 9	17 ± 12	28 ± 18	17 ± 10	0.04	0.77
ICS-untreated period, years	5 ± 6	8 ± 11	18 ± 20	8 ± 9	0.08	0.90
ICS daily maintenance dose, μg†	483 ± 291	525 ± 305	475 ± 314	763 ± 402	0.04	0.04
No. of other controller medications	1.3 ± 1.1	1.0 ± 1.2	0.8 ± 0.8	1.8 ± 1.3	0.02	0.02
Treatment step 5, %‡	3	3	0	22	0.004	0.03
Asthma control test (points)	23.2 ± 2.1	23.6 ± 2.5	23.0 ± 3.7	23.1 ± 2.1	0.32	0.12
Serum IgE, IU/mL	112 (0–1300)	298 (0–2090)	212 (10–3740)	233 (27–16,000)	0.15	0.20
Atopy, n (%)	77	76	68	57	0.31	0.11
WBC, cells/μL	5638 ± 1516	6121 ± 1151	5780 ± 1387	5961 ± 1484	0.34	0.41
Eosinophils, cells/μL	164 ± 145	322 ± 170	289 ± 383	385 ± 265	<0.0001	0.71
Neutrophil, cells/μL	3436 ± 1017	3694 ± 969	3364 ± 1107	3613 ± 1469	0.43	0.26
FeNO, ppb	17.1 ± 4.3	52.2 ± 34.3	18.9 ± 4.1	61.9 ± 31.0	<0.0001	0.16
Serum periostin, ng/mL	71.8 ± 17.9	74.1 ± 11.9	118.2 ± 20.9	135.8 ± 44.0	<0.0001	–
History of admission due to asthma, %	13	12	36	39	0.01	0.02
FEV_1 at enrollment, % predicted	107 ± 18	99 ± 16	99 ± 29	101 ± 19	0.17	0.57
Annual changes in FEV_1, mL/year	2.7 ± 25.9	12.5 ± 37.1	1.9 ± 22.0	−19.1 ± 43.1	0.047§	0.003‖
Rapid decliner, n (%)¶	3 (8)	2 (6)	3 (12)	8 (35)	0.007§	0.01‖
Mean (±SD) number of asthma exacerbations per patient in the 2 subsequent years	0.31 ± 0.83	0.21 ± 0.48	0.83 ± 2.51#	0.57 ± 0.79	0.18	0.05
POSTN rs3829365, GG (%)	23	56	50#	52	0.02	0.78
IL4RA rs8832, GG (%)	15	12	21#	35	0.16	0.04

Results are presented as means ± SD, except for IgE [medians (ranges)]. FeNO, exhaled nitric oxide; ICS, inhaled corticosteroids; IgE, immunoglobulin E; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity.
*P values among 4 groups. **P values between groups B and D.
† Equivalent to fluticasone propionate.
‡ According to the Global Initiative for Asthma 2010 guideline.
§ Crude analysis without adjustment with sex, height, age at enrollment, and FEV_1 at the first measurement.
¶ Rapid decliners were defined as patients with a decline in $FEV_1 \geq 30$ mL per year.
‖ Adjusted by sex, height, age at enrollment, and FEV_1 at the first measurement.
Missing in one patient.

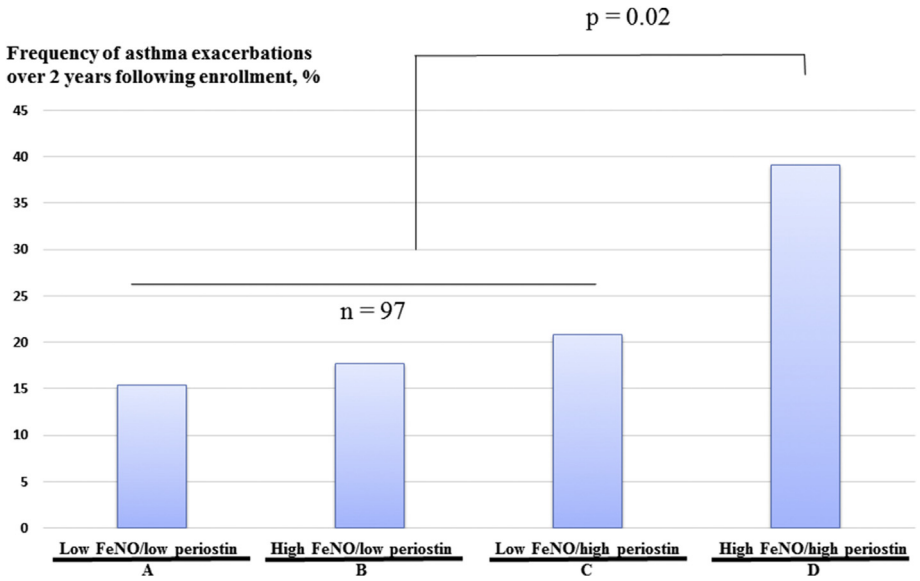

Fig. 2. Frequency of asthma exacerbations over 2 years following enrollment in patients belonging to the four groups stratified according to FeNO and serum periostin levels.

longest disease duration (Fig. 3e) and ICS-untreated period, with a gap of approximately 10 years among the four groups. Lastly, even when the cutoff value of FeNO was set at 40 ppb for analysis, this level was shown to be appropriate to identify patients with poorly controlled asthma[57] and patients with treated asthma with a more rapid decline in FEV_1.[23] The following aspects of the four groups remained significantly different: higher frequencies of the Global Initiative for Asthma (GINA) treatment step 5, subsequent asthma exacerbations, and more rapid declines in group D than in group B; taller and lower frequencies of history of admission due to asthma

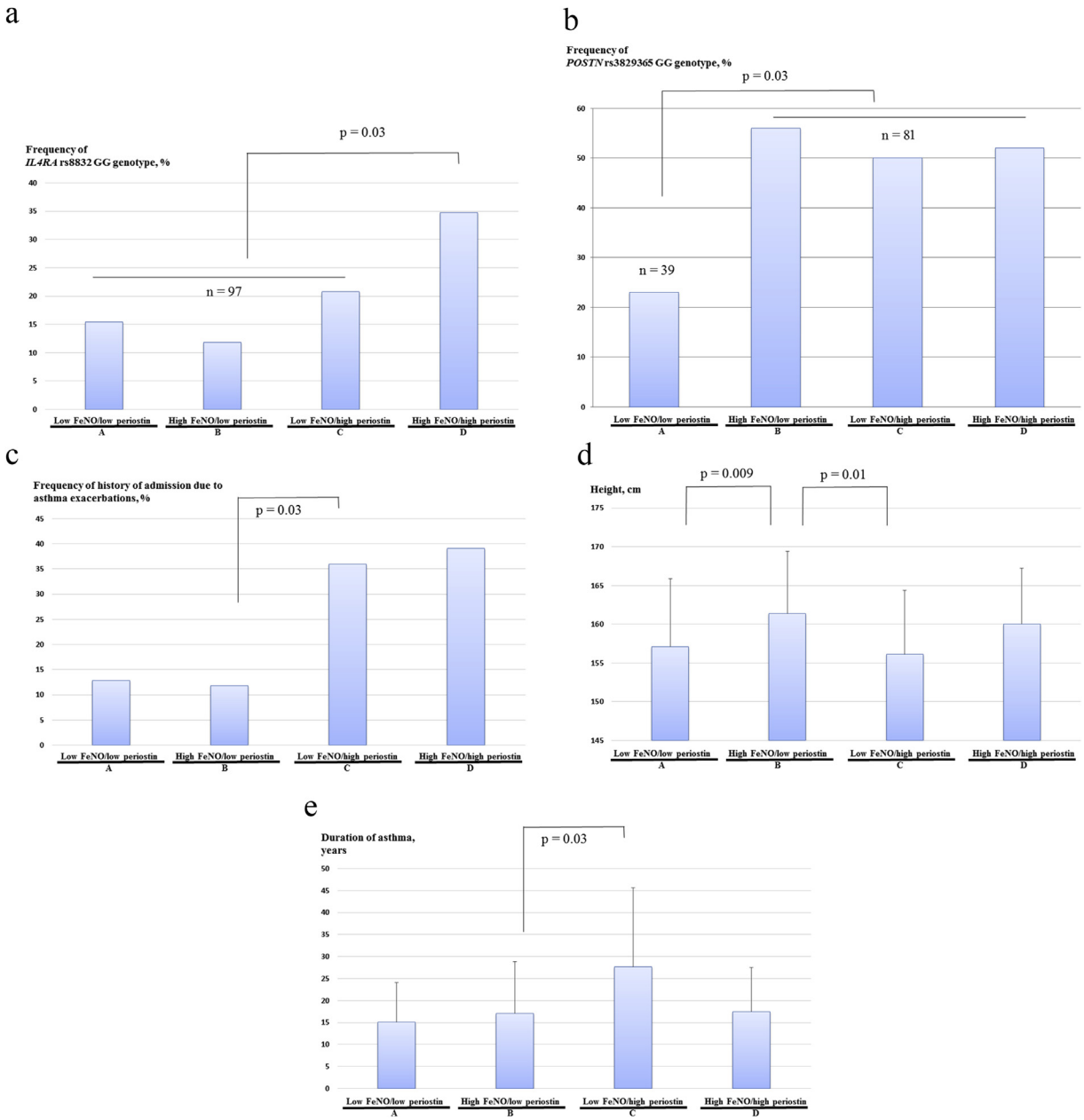

Fig. 3. **(a)** Frequency of *IL4RA* rs8832GG genotype, **(b)** frequency of *POSTN* rs3829365GG genotype, **(c)** frequency of history of admission due to asthma exacerbations, **(d)** height, **(e)** duration of asthma in the four groups, stratified according to FeNO and serum periostin levels. In **(c)**–**(e)**, $P < 0.05$ was considered significant for comparison between B and C, which was our main interest; using the Bonferroni correction, $P < 0.01$ was considered significant for comparison between the other two groups. In one patient in group C, the same patient who was lost to follow-up, variants of *IL4RA* and *POSTN* genes could not be analyzed because of insufficient DNA quality.

in group B than in group C; the longest disease duration and ICS-untreated period in group C; the least frequent GG genotype of *POSTN* rs3829365 in group A; and the highest frequency of the GG genotype of *IL4RA* rs8832 in group D (data not shown).

Conclusively, high levels of both FeNO and serum periostin may reflect severe type-2/eosinophilic airway inflammation. However, each biomarker has specific characteristics and modifiers; patients with either high FeNO or serum periostin levels only should be treated with ICS but may not necessarily require as intense treatment as patients with high levels of both markers.

Conclusions

Because patients with severe eosinophilic inflammation do not always complain of symptoms of asthma,[58,59] the identification of patients at risk of asthma exacerbations and pulmonary function decline is clinically important. The use of a composite marker of FeNO and serum periostin levels may have utility in achieving this goal.

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Conflict of interest

TN received research funding from GlaxoSmithKline. HM received research funding from GlaxoSmithKline; and lecture fees from AstraZeneca, Novartis Pharma, and Boehringer Ingelheim. KI received research funding from Chugai Pharmaceutical and Shino-Test; honoraria for AstraZeneca; and advisory role in Chugai Pharmaceutical.

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