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

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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 asthma4 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...
either high FeNO levels or serum periostin 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. 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
NOS (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,
dyspnea.11–13 Elevated FeNO levels predict good responses to
inhaled corticosteroid (ICS) treatment, particularly in ICS-naïve
patients with asthma.14–16 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.11,19 Elevated FeNO levels also
reflect oxidative/nitrative stress in the airways, which drives fibrosis
progression20 and may represent a marker of excess decline in pul-
monary function when sufficiently elevated.21–23 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 pro-
longed 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 airways28; and activates functions
of eosinophils, including O2 generation.33 Thus, high serum peri-
ostin 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,25,29 and may predict treatment failure while tapering
ICS doses35 and good responses to biologics against type-2 pathway
in patients with asthma.26,33 In contrast with FeNO, serum periostin
levels are stable with a small coefficient of variation40,41 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, serum periostin levels are
elevated in fibrotic diseases, such as idiopathic interstitial pneu-
monia26 and scleroderma47 (Table 1).

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 sensi-
tivity 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 eosino-
philia 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; FEV1, 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; FEV1, 100%; daily
ICS doses, 500 μg fluticasone propionate equivalent).38 However,
the potential mechanisms underlying this discrepancy may be
attributable to differences in periostin assay systems and disease
severity among studied patients.45 A recent study of Japanese pa-
ediatric 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 Alz-
heimer’s disease,52 and severe graft-versus-host disease,32 the su-
niority 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 per-
iosis in predicting severe eosinophilic asthma have been reported.

In a sub-analysis of the Kinki Hokuriku Airway disease Confer-
ence (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 1
Characteristics of FeNO and serum periostin.

<table>
<thead>
<tr>
<th>Feature</th>
<th>FeNO</th>
<th>Serum periostin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Relevant cytokines</strong></td>
<td>IL-4</td>
<td>IL-10, IL-15</td>
</tr>
<tr>
<td><strong>Modifiers</strong></td>
<td></td>
<td>Idiopathic pulmonary</td>
</tr>
<tr>
<td>Height</td>
<td>+</td>
<td>TGF-β21,27</td>
</tr>
<tr>
<td>Male gender</td>
<td></td>
<td>Fibrosis 1,46</td>
</tr>
<tr>
<td>Nitrate-rich diet</td>
<td></td>
<td>Scleroderma 47</td>
</tr>
<tr>
<td>Airway viral infection</td>
<td></td>
<td>Bone marrow fibrosis 73</td>
</tr>
<tr>
<td>Current smoking</td>
<td></td>
<td>Proliferative diabetic</td>
</tr>
<tr>
<td>Spirometric manoeuvres</td>
<td></td>
<td>Retinopathy 1,44</td>
</tr>
<tr>
<td>Atopic predisposition</td>
<td>+</td>
<td>Non-alcoholic fatty liver</td>
</tr>
<tr>
<td>Allergic rhinitis</td>
<td>+</td>
<td>IgG4-related diseases 76</td>
</tr>
<tr>
<td>Responsiveness to ICS</td>
<td>+</td>
<td>Atopic dermatitis 47</td>
</tr>
<tr>
<td>Pulmonary function decline</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

67; 32 males; mean age, 46 years; FEV1, 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; FEV1, 100%; daily ICS doses, 500 μg fluticasone propionate equivalent).38 However, the potential mechanisms underlying this discrepancy may be attributable to differences in periostin assay systems and disease severity among studied patients.45 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.

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ence (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
chemiluminescence analyzer (NOA 280, Sievers, Boulder, CO, USA), according to the American Thoracic Society (ATS) guidelines. 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. 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. Because periostin expression is upregulated with the stimulation of IL-4 and IL-13 and high serum periostin levels strongly reflect airway eosinophilic inflammation, 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).

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. Patients in group D (n = 23) received more intensive treatment, had a history of asthma admission, and a decline in FEV1 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 (FEV1 < 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 POSTN rs3829365 that was associated with elevated serum periostin levels was the least frequent in group A (low levels of both FeNO and periostin; 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 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
controlled asthma and patients with treated asthma with a more
level was shown to be appropriate to identify patients with poorly
when the cutoff value of FeNO was set at 40 ppb for analysis, this
gap of approximately 10 years among the four groups. Lastly, even
longest disease duration (Fig. 3e) and ICS-untreated period, with a
frequency of asthma exacerbations over 2 years following enrollment in patients belonging to the four groups stratified according to FeNO and serum periostin levels. 

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Low FeNO/low periostin</th>
<th>High FeNO/low periostin</th>
<th>Low FeNO/high periostin</th>
<th>High FeNO/high periostin</th>
<th>P value*</th>
<th>P value**</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (n = 39)</td>
<td>33/6</td>
<td>19/15</td>
<td>19/6</td>
<td>17/6</td>
<td>0.05</td>
<td>0.26</td>
</tr>
<tr>
<td>B (n = 34)</td>
<td>56 ± 14</td>
<td>59 ± 12</td>
<td>63 ± 13</td>
<td>60 ± 12</td>
<td>0.23</td>
<td>0.90</td>
</tr>
<tr>
<td>C (n = 25)</td>
<td>41 ± 16</td>
<td>42 ± 18</td>
<td>35 ± 19</td>
<td>42 ± 16</td>
<td>0.46</td>
<td>0.99</td>
</tr>
<tr>
<td>D (n = 23)</td>
<td>157 ± 9</td>
<td>161 ± 8</td>
<td>156 ± 8</td>
<td>160 ± 7</td>
<td>0.01</td>
<td>0.52</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>23.6 ± 3.6</td>
<td>23.7 ± 2.7</td>
<td>22.5 ± 3.1</td>
<td>22.1 ± 2.2</td>
<td>0.24</td>
<td>0.02</td>
</tr>
<tr>
<td>Smoking history, ex (%)</td>
<td>23</td>
<td>24</td>
<td>20</td>
<td>26</td>
<td>0.97</td>
<td>0.83</td>
</tr>
<tr>
<td>Disease duration, years</td>
<td>15 ± 9</td>
<td>17 ± 12</td>
<td>28 ± 18</td>
<td>17 ± 10</td>
<td>0.04</td>
<td>0.77</td>
</tr>
<tr>
<td>ICS-untreated period, years</td>
<td>5 ± 6</td>
<td>8 ± 11</td>
<td>18 ± 20</td>
<td>8 ± 9</td>
<td>0.08</td>
<td>0.90</td>
</tr>
<tr>
<td>ICS daily maintenance dose, µg</td>
<td>483 ± 291</td>
<td>525 ± 305</td>
<td>475 ± 314</td>
<td>763 ± 402</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>No. of other controller medications</td>
<td>1.3 ± 1.1</td>
<td>1.0 ± 1.2</td>
<td>0.8 ± 0.8</td>
<td>1.6 ± 1.3</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>Treatment step 5, %</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>22</td>
<td>0.004</td>
<td>0.03</td>
</tr>
<tr>
<td>Asthma control test (points)</td>
<td>23.2 ± 2.1</td>
<td>23.6 ± 2.5</td>
<td>23.0 ± 3.7</td>
<td>23.1 ± 2.1</td>
<td>0.32</td>
<td>0.12</td>
</tr>
<tr>
<td>Serum IgE, IU/mL</td>
<td>112 (0–1300)</td>
<td>298 (0–2090)</td>
<td>212 (10–3740)</td>
<td>233 (27–16000)</td>
<td>0.15</td>
<td>0.20</td>
</tr>
<tr>
<td>Atopt, n (%)</td>
<td>77</td>
<td>76</td>
<td>68</td>
<td>57</td>
<td>0.31</td>
<td>0.11</td>
</tr>
<tr>
<td>WBC, cells/µL</td>
<td>5638 ± 1516</td>
<td>6121 ± 1151</td>
<td>5780 ± 1387</td>
<td>5961 ± 1484</td>
<td>0.34</td>
<td>0.41</td>
</tr>
<tr>
<td>Neutrophils, cells/µL</td>
<td>164 ± 145</td>
<td>322 ± 170</td>
<td>289 ± 383</td>
<td>385 ± 265</td>
<td>&lt;0.0001</td>
<td>0.71</td>
</tr>
<tr>
<td>Neutrophil, cells/µL</td>
<td>3436 ± 1017</td>
<td>3694 ± 969</td>
<td>3364 ± 1107</td>
<td>3613 ± 1469</td>
<td>0.43</td>
<td>0.26</td>
</tr>
<tr>
<td>FeNO, ppb</td>
<td>17.1 ± 4.3</td>
<td>52.2 ± 34.3</td>
<td>18.9 ± 4.1</td>
<td>61.9 ± 31.0</td>
<td>&lt;0.0001</td>
<td>0.16</td>
</tr>
<tr>
<td>Serum periostin, ng/mL</td>
<td>71.8 ± 17.9</td>
<td>74.1 ± 11.9</td>
<td>118.2 ± 20.9</td>
<td>135.8 ± 44.0</td>
<td>&lt;0.0001</td>
<td>–</td>
</tr>
<tr>
<td>History of admission due to asthma, %</td>
<td>13</td>
<td>12</td>
<td>36</td>
<td>39</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>FEV₁ at enrollment, % predicted</td>
<td>107 ± 18</td>
<td>99 ± 16</td>
<td>99 ± 29</td>
<td>101 ± 19</td>
<td>0.17</td>
<td>0.57</td>
</tr>
<tr>
<td>Annual change in FEV₁, mL/year</td>
<td>2.7 ± 25.9</td>
<td>12.5 ± 37.1</td>
<td>1.9 ± 22.0</td>
<td>−19.1 ± 43.1</td>
<td>0.047</td>
<td>0.003</td>
</tr>
<tr>
<td>Rapid decliner, n (%)</td>
<td>3 (8)</td>
<td>2 (6)</td>
<td>3 (12)</td>
<td>8 (35)</td>
<td>0.007</td>
<td>0.01</td>
</tr>
<tr>
<td>Mean (±SD) number of asthma exacerbations per patient in the 2 subsequent years</td>
<td>0.31 ± 0.83</td>
<td>0.21 ± 0.48</td>
<td>0.83 ± 2.51</td>
<td>0.57 ± 0.79</td>
<td>0.18</td>
<td>0.05</td>
</tr>
<tr>
<td>POSTN rs3829365, GG (%)</td>
<td>23</td>
<td>56</td>
<td>50</td>
<td>52</td>
<td>0.02</td>
<td>0.78</td>
</tr>
<tr>
<td>IL1RA rs883232, GG (%)</td>
<td>15</td>
<td>12</td>
<td>21</td>
<td>35</td>
<td>0.16</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Results are presented as means ± SD, except for IgE [medians (ranges)]. FeNO, exhaled nitric oxide; ICS, inhaled corticosteroids; IgE, immunoglobulin E; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity.

* P values among 4 groups. **P values between groups B and D.
1 According to the Global Initiative for Asthma 2010 guideline.
2 Crude analysis without adjustment with sex, height, age at enrollment, and FEV₁ at the first measurement.
3 Rapid decliners were defined as patients with a decline in FEV₁ ≥ 30 mL per year.
4 Adjusted by sex, height, age at enrollment, and FEV₁ 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.
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, 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.

Acknowledgement

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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.
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.

References
