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Original Article

Usefulness of serum biopterin as a predictive biomarker for childhood asthma control: A prospective cohort study

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AR, allergic rhinitis; BA, bronchial asthma; BH4, tetrahydrobiopterin; ELISA, enzyme-linked immunosorbent assay; GINA, global initiative for asthma; ICS, inhaled corticosteroid; JPAC, Japanese pediatric asthma control program; LTRA, leukotriene receptor antagonists; NOS, nitric oxide synthase; TlgE, total immunoglobulin E

ABSTRACT

Background: Pteridines are metabolites of tetrahydrobiopterin, which serves as co-enzyme of nitric oxide synthase. We sought to investigate the usefulness of pteridines as biomarkers for childhood asthma control.

Methods: We conducted a single-center prospective cohort study involving 168 asthmatic children aged 4–17 years who visited the periodical asthma checkup program. Serum neopterin and biopterin levels were measured as pteridines at each visit along with measurement of FeNO, respiratory function tests, nasal eosinophil test, blood eosinophil count, and IgE level. We calculated coefficients for relation between pteridines and asthma control, which was assessed by questionnaires (JPAC: Japanese Pediatric Asthma Control Program).

Results: A total of 168 participants aged 10.3 ± 3.39 years (mean $\pm$ SD) with asthma were recruited. The participants in this study contained 58 patients (34.5%) of complete-controlled based on JPAC, 132 patients (76.0%) of well-controlled group based on GINA. FeNO and serum neopterin level did not correlate with following period's JPAC scores. In contrast, serum biopterin level significantly correlated with following period's JPAC total score (Coefficients 0.398; 95% CI 0.164 to 0.632; p value 0.001) and frequency of wheezing during exercise (Coefficients 0.272; 95% CI 0.217 to 0.328; p value < 0.001).

Conclusions: We found serum biopterin effected the following period's control status of asthmatic children, thus monitoring biopterin level will be a useful for management of asthma to adjust treatment.

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Introduction

Controlling airway chronic inflammation is mainstay of asthma treatment. Therefore, monitoring airway inflammation is essential for clinical management. Several biomarkers have been developed to estimate the degree of airway inflammation.¹ Fractional exhaled nitric oxide (FeNO) is widely used for diagnosis and management of asthma over the last decade.^{2,3} FeNO had been proven to reflect airway eosinophilic inflammation and responsiveness to corticosteroid.^{4–7}

Measurement of FeNO, however, encounters the following problems especially for children; 1) the values are influenced by several comorbidities such as allergic rhinitis, 2) inhaled corticosteroids (ICS) rapidly lower FeNO in patients especially with atopic asthma,^{4,8,9} 3) the correlation between FeNO and asthma control are inconsistent.^{3,9,10} Thus, there is a need to develop a biomarker which strongly correlates with asthma control status.

Previously, we demonstrated that levels of serum neopterin and biopterin were inversely correlated with FeNO in asthmatic children.¹¹ Neopterin and biopterin are called pteridines, both of them are intermediate metabolites during tetrahydrobiopterin (BH4) synthesis pathway.¹² BH4 serves as a cofactor for every nitric oxide synthase (NOS) to produce nitric oxide (NO) from L-arginine.^{12,13} Serum neopterin level is known to be increased by stimulation of Th1-type cytokines such as interferon- γ (IFN- γ), tumor necrosis factor alpha (TNF- α), IL-2 when people have viral infections or other inflammatory

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disease conditions.^{14–16} Serum biopterin level directly reflects systemic pool of BH₄. Insufficiency of BH₄ during NO synthesis will cause NOS uncoupling, which induce superoxide generation.^{17,18}

Based on previously reported data from our group¹¹ and several biochemical evidences from others,^{12,13,17,18} we hypothesize that lowering levels of serum pteridines will have a risk of asthma control failure. To test this hypothesis, we prospectively analyzed the relation between pteridines and asthma control status in 168 children with asthma.

Methods

Study design and subjects recruitment

A total of 168 asthmatic children (4–17 years) were recruited from periodical asthma checkup program 2014–2016 at Osaka City University Hospital. All patients were diagnosed with asthma by using the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire at the first visit. These children were followed prospectively four times a year for each season. At each visit, we performed the followings; medical examination by pediatricians, objective questionnaire about last acute or sub-acute asthma exacerbation and treatment to assess asthma control status in accordance with Japanese Pediatric Asthma Control Program (JPAC), respiratory function tests (FeNO, spirometry, airway resistance), nasal eosinophil test, and blood test. Children with infectious symptom such as fever, vomit, diarrhea, and received systemic treatment with corticosteroids were excluded.

Ethics statement

This study was approved by the Institutional Review Boards at Osaka City University Graduate School of Medicine (No. 3021). Written informed consent was obtained from parents or guardians, and children were asked for their assent.

Evaluation of asthma severity and control status

Each patient's asthma severity was categorized into intermittent, mild persistent, moderate persistent, and severe persistent according

Japanese Pediatric Asthma Control Program (JPAC) questionnaire

Answer the following questions 1–5 about asthma symptoms and troubles in daily life in the last month. Check the appropriate boxes about frequency.

1. How many days did wheezing occur in the last month?

Not at all (3)	Once or more a month, less than once a week (2)	Once or more a week, not everyday (1)	Continued everyday (0)
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2. How often did dyspnea (choking) occur by asthma attacks in the last month?

Not at all (3)	Occasionally occurred and not persisted (2)	Frequently occurred and persisted (1)	Continued almost everyday (0)
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3. How many days did you awake because of asthma attacks at night in the last month?

Not at all (3)	Occasionally, less than once a week (2)	Once or more a week, not everyday (1)	Everyday (0)
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4. Are you troubled with wheezing during exercise?

Not at all (3)	Slightly (2)	Frequently (1)	Always (0)
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5. How many days did you use an inhalant, oral medicine or patch for asthma attacks in the last one month?

Not at all (3)	Less than once a week (2)	Frequently (1)	Always (0)
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Scoring is as follows: total 15 points is complete control; 12–14 is favorable (insufficient) control; and ≤ 11 is poor control.

Fig. 1. Japanese Pediatric Asthma Control Program (JPAC) questionnaire. Modified from the web page of Japanese Society of Pediatric Allergy and Clinical Immunology with permission (http://www.jspaci.jp/modules/journal/index.php?content_id=13). Scoring is as follows: total 15 points is complete control, 12–14 is favorable (insufficient) control, and ≤ 11 is poor control.

to Global Initiative for Asthma (GINA) 2014 and Japanese pediatric guideline for treatment and management of bronchial asthma (JPGL) 2012 respectively. In JPGL 2012, we determined the severity as “true severity” considering the treatment steps at the patient's first visit. To assess asthma control status, we used both GINA guideline and JPAC questionnaire (Fig. 1). In this study, JPAC questionnaire was conducted scoring by trained pediatricians. JPAC score had been validated to correlate with Childhood Asthma Control Test (C-ACT) score, %FEV₁ and %MMF.^{19,20} Using JPAC questionnaire, we can assess the severity and control status of asthmatic children and select asthma treatment step according to Japanese pediatric guideline for treatment and management of bronchial asthma (JPGL) 2012 guideline. JPAC questionnaire consists of 5 items; to assess the frequency of wheezing (Question 1; Q1), dyspnea by acute asthma exacerbation (Question 2; Q2), night awaking because of asthma (Question 3; Q3), wheezing during exercise (Question 4; Q4) and the use of rescue medications (Question 5; Q5) with 4-week recall on symptoms and daily functioning. Each question is scaled by 0–3 points (0 = frequency of symptom continued everyday/uncontrolled; 1 = frequency of symptom is once or more a week, not everyday; 2 = frequency of symptom is once or more a month, less than once a week; 3 = frequency of symptom is not at all/well-controlled). Total score of 5 questions (range 0–15) is defined as JPAC score; 15 points classified as complete control, 12–14 points is favorable (insufficient) control, and ≤ 11 points is poor control.²¹ At each visit, participants had another questionnaire about the frequency and severity of acute or sub-acute asthma exacerbations during two visits.

Pteridines analysis

Measurement of serum pteridines was performed by high performance liquid chromatography (HPLC, SHIMADZU LC-10) as previously described.²²

Clinical laboratory test

Peripheral blood eosinophil cell counts were performed in each patient and an automated hematology analyzer XN (Sysmex, Kobe, Japan) determined the blood eosinophil percentage. Total nonspecific and specific serum IgE (mite, cat, dog, grass) were measured by ImmunoCAP™ (Thermo Fisher Diagnostics, Tokyo, Japan). Atopic asthma was defined as having ≥ 0.35 kU/l specific IgE level concentration ($\geq$ CAP class 1).

Pulmonary function tests

Pulmonary function tests were performed using a spirometer, CHESTGRAOH HI-105T (Chest M.I., Osaka, Japan) according to the criteria of the American Thoracic Society (ATS)/European Respiratory Society and the Japanese Respiratory Society. Airway resistance was measured with an impulse oscillometry (IOS), Master Screen IOS-J. (E. Jaeger GmbH, Wurzburg, Germany). FeNO level was measured using NIOX VERO devices (Aerocrine AB, Sweden) according to the manufacturer's instructions and the ATS guidelines.⁴

Statistical analysis

Patients' baseline characteristics were summarized using medians and interquartile ranges for continuous variables whereas counts and percentage for categorical variables.

In order to assess the effects of serum biopterin on each JPAC score (Total, Q1, Q2, Q3, Q4, Q5), we conducted a multivariable linear regression analysis with the Huber-White robust sandwich estimator of variance-covariance matrix. The robust estimator accounts for dependence in repeated measures. This model included

covariates indicating sex, age, a use of ICS, LTRA and/or nasal corticosteroid, diagnosis of asthma, the counts of the examination time, a season and JPAC score measured on the preceding examination. Similar analysis to above was conducted to evaluate the effect of serum neopterin on each JPAC score. Furthermore, we examined the effect of FeNO on JPAC score. The similar regression model to the above was conducted with a variable indicating FeNO. As sensitivity analysis, multivariable linear regression models were conducted with the cross-product term to assess whether the association between bioppterin and next JPAC score was modified by seasons and also between bioppterin and asthma severities.

All statistical inferences were conducted with 2-sided significance level of 5% using R software version 3.3.1 (<https://www.r-project.org/foundation/>) with the “rms” package.

Results

Baseline characteristics

A total of 168 patients between 4 and 17 years with asthma were included in the study (Table 1). Their mean age was 10.3 years. 159 patients (94.7%) were categorized as atopic asthma. Regarding with asthma control status of the participants, 58 patients (34.5%) were classified as complete-controlled based on JPAC. 132 patients (76.0%) were considered as well-controlled group when we applied GINA classification. The mean total JPAC score of the participants was 13.3 ± 2.17 SD (max. score 15). The mean question 4 score of JPAC, “frequency of wheezing during exercise”, was 2.3 (max. score 3), which was lower than other items. In terms of treatment intensity for long-term asthma management, 121 patients (72%) were treated with GINA treatment step 1, where ICS therapy was not required. 45 patients (27.8%) were receiving ICS therapy: 12 Fluticasone propionate (50–200 µg daily), 4 Beclomethasone dipropionate (50–200 µg daily), 4 Ciclesonide (100–200 µg daily), 5 Budesonide (50–500 µg daily), 4 Budesonide (nebulizer; 250–500 µg daily), and 16 Salmeterol/Fluticasone (50–500 µg daily). 99 patients (59.0%) were receiving LTRA. One patient received theophylline.

The results of laboratory and lung function test at the first visit are summarized in Table 2. The median total IgE level was 306.5 IU/l (IQR 95.9, 599). 159 patients (94.7%) were sensitized towards at least one of mite, cat, dog, and grass to produce specific IgE. Both serum neopterin and bioppterin levels of the participants tended to be lower than previously reported range from healthy control children (Neopterin: 17.9 ± 10.6 , Bioppterin: 8.2 ± 5.9 , in children).²³ Lung function was stable where %FEV1 and %VC did not show any signs of active exacerbation. The median FeNO was 22 ppb, but 49 patients (29.2%) were above 35 ppb which was clinically significant cut points for FeNO in children.⁴

The impact of FeNO on the assessment of asthma control

FeNO is often measured to monitor asthma control status. Therefore, We analyzed coefficients for following period's JPAC score, the frequency of asthma exacerbation associated with FeNO by using logistic regression model (Table 3, Supplementary Fig. 1). Based on this regression model, FeNO did not show a significant impact on following period's JPAC total score (Coefficients 0.025; 95% CI –0.104 to 0.153; *p* value = 0.708).

The impact of serum neopterin on the assessment of asthma control

We also calculated coefficients for following period's JPAC score, the frequency of asthma exacerbation associated with neopterin by using logistic regression model (Table 3, Supplementary Fig. 2).

Table 1
Patients' characteristics.

	n = 168
Age, years, mean ($\pm$ SD)	10.3 ($\pm$ 3.39)
Male, sex, n (%)	96 (57.1)
Allergic rhinitis, n (%)	100 (75.8)
Allergic conjunctiva, n (%)	43 (32.6)
Food allergy, n (%)	49 (37.1)
Allergic dermatitis, n (%)	17 (12.9)
Atopic asthma, n (%)	159 (94.7)
Asthma severity (GINA)	
Intermittent, n (%)	142 (84.5)
Mild persistent, n (%)	26 (15.5)
Moderate persistent, n (%)	0 (0)
Severe persistent, n (%)	0 (0)
Asthma severity (JPGL)	
Intermittent, n (%)	69 (41.1)
Mild persistent, n (%)	46 (27.5)
Moderate persistent, n (%)	19 (11.4)
Severe persistent, n (%)	33 (19.8)
Most severe persistent, n (%)	1 (0.6)
Asthma symptom control level (GINA)	
Well-controlled, n (%)	132 (78.6)
Partly-controlled, n (%)	31 (18.5)
Poor-controlled, n (%)	5 (3.0)
Asthma symptom control status (JPAC)	
Complete-controlled, n (%)	58 (34.5)
Favorable-controlled, n (%)	74 (44.0)
Poor-controlled, n (%)	34 (20.2)
JPAC score, median (IQR)	
JPAC total score	14.0 (13.00,15.00)
JPAC Q1 (Frequency of wheezing)	3.0 (2.25,3.00)
JPAC Q2 (Frequency of dyspnea by asthma exacerbation)	3.0 (3.00,3.00)
JPAC Q3 (Frequency of night awaking because of asthma)	3.0 (3.00,3.00)
JPAC Q4 (Frequency of wheezing during exercise)	2.0 (2.00,3.00)
JPAC Q5 (The use of rescue medications)	3.0 (3.00,3.00)
Use of ICS, n (%)	45 (26.8)
Use of LTRA, n (%)	99 (59.0)
Use of nasal corticosteroid, n (%)	4 (2.4)
GINA treatment	
Step 1	121 (72.0)
Step 2	29 (17.3)
Step 3	15 (8.9)
Step 4	3 (1.8)

Asthma severities of JPGL were showed as true severity considering the treatment step. Asthma symptom control levels were defined using by JPAC (0–11: poor-controlled, 12–14: favorable-controlled, 15: complete-controlled). Results are provided as mean $\pm$ SD or absolute number with percent age (%). SD, standard deviation; GINA, Global Initiative for Asthma; JPAC, Japanese Pediatric Asthma Control Program; JPAC Q1–Q5, question 1–5 of JPAC questionnaire; ICS, inhaled corticosteroid; LTRA, leukotriene receptor antagonist.

Serum neopterin showed no significant correlation with the following period's JPAC score or severity of asthma exacerbation. Each item of JPAC and severity of asthma exacerbation demonstrated no significant association between serum neopterin.

Table 2
Laboratory test and Lung function at first visit.

	n = 168
Blood eosinophils (/ml), median, (IQR)	310 (180,480)
Total IgE (IU/l), median, (IQR)	306.5 (95.9,599)
Nasal eosinophil positive, n (%)	90 (54.9)
Serum periostin (ng/ml), median, (IQR)	94.5 (81.0,112.3)
Serum neopterin (nmol/l), median, (IQR)	6.4 (4.3,9.8)
Serum bioppterin (nmol/l), median, (IQR)	4.8 (3.8,6.2)
VC (%), median, (IQR)	94.4 (83.1,103.6)
FEV ₁ pred (%), median, (IQR)	84.4 (79.0,90.9)
FeNO (ppb), median, (IQR)	22 (13,38)
R5–R20, median, (IQR)	0.15 (0.06,0.24)
X at 5 Hz, median, (IQR)	–0.18 (–0.23,–0.11)

Pred %, predicted %; R5, resistance at oscillation frequency of 5 Hz; R20, resistance at oscillation frequency of 20 Hz; $\times$ 5, reactance at oscillation frequency of 5 Hz; IQR, Interquartile range.

Table 3
Impact of potential biomarkers on JPAC score and attack frequency.

	Coefficients	Lower 95% CI	Upper 95% CI	p value
FeNO				
Next JPAC total score	0.025	−0.104	0.153	0.708
Attack frequency	0.954	0.697	1.305	0.767
Neopterin				
Next JPAC total score	0.298	−0.100	0.697	0.141
Attack frequency	0.265	0.046	1.544	0.140
Biopterin				
Next JPAC total score	0.398	0.164	0.632	0.001
Attack frequency	0.059	0.000	56.421	0.420

Attack frequency: the frequency of acute or sub-acute asthma exacerbations during two visits. Coefficients for JPAC score and attack frequency by serum neopterin and biopterin change per 10 nmol/l, FeNO change per 10 ppb.

JPAC, Japanese Pediatric Asthma Control Program; JPAC Q1–Q5, question 1–5 of JPAC questionnaire.

The impact of serum biopterin on the assessment of asthma control

Similarly, we calculated coefficients for following period's JPAC score, the frequency and severity of asthma exacerbation associated with biopterin by using logistic regression (Table 3, Supplementary Fig. 3). Serum biopterin significantly correlated with the following period's JPAC total score (Coefficients 0.398; 95% CI 0.164 to 0.632; p value = 0.001). The median period of following period from previous visit was 112 day (IQR 76,146). Based on this regression model, JPAC score increased 0.398 per 10 nM biopterin increase. However, there were no relations between frequency of asthma exacerbation and serum biopterin. Since asthma control status varies by season, we further assessed whether seasons affect the relation between JPAC score and serum biopterin. The slope of biopterin assessed in autumn had a little bit of different trend, though there was no significant interaction between biopterin and seasons (p for interaction = 0.192) (Supplementary Fig. 4).

To evaluate the association between biopterin and each asthma symptom, we calculated coefficients for each JPAC questionnaire associated with serum biopterin. The effect of serum biopterin on JPAC Q4 showed strong correlation (Coefficients 0.272; 95% CI 0.217 to 0.328; p value < 0.001) (Table 4).

The impact of serum biopterin according to asthma severity

To explore the usefulness of biopterin for assessing asthma control, we calculated the impact of asthma severities on the relation between serum biopterin and asthma control. We assessed the interaction of severities based on both GINA and JPGL classification. In GINA classification, there was a significant interaction between biopterin and asthma severities (p for interaction = 0.013) (Supplementary Fig. 5a). The mild group tended to have more impact of biopterin than intermittent group on JPAC score. Similarly, a significant interaction was found between biopterin and asthma severities in JPGL classification (p for interaction = 0.027) (Supplementary Fig. 5b). The more severe group tended to have more impact of biopterin on JPAC score.

Discussion

As the main result of this prospective study, we identified that serum biopterin correlated to the state of the following period's asthma control in children with stable asthma. Therefore, biopterin has a potential to serve as a predictive biomarker for asthma control, especially in children who have difficulty in measuring FeNO. Monitoring serum biopterin level during the follow up may be able

Table 4
The effects for each following period's JPAC questionnaire associated with serum biopterin.

	Coefficients	Lower 95% CI	Upper 95% CI	p value
JPAC Q1 (Frequency of wheezing)	0.117	0.037	0.198	0.005
JPAC Q2 (Frequency of dyspnea by asthma exacerbation)	0.015	−0.046	0.075	0.634
JPAC Q3 (Frequency of night awaking because of asthma)	−0.035	−0.092	0.022	0.228
JPAC Q4 (Frequency of wheezing during exercise)	0.272	0.217	0.328	0.000
JPAC Q5 (The use of rescue medications)	0.062	−0.045	0.168	0.254

Coefficients for each JPAC score by serum biopterin change per 10 nmol/l.

JPAC, Japanese Pediatric Asthma Control Program; JPAC Q1–Q5, question 1–5 of JPAC questionnaire.

to assess asthma control status and to adjust treatment intensity more precisely.

In this study, the high proportion (94.7%) of atopic asthma found in this study was consistent with the prevalence among urban asthmatic children.²⁴ Baseline characteristics also showed high rate of participants' asthma severities were classified as intermittent and mild based on GINA. This means that relatively mild asthmatic children were recruited in this study. Compared to GINA classification, asthma control levels classified by JPAC defined much poorer-controlled patients. Therefore, JPAC might be highly sensitive to assess mild asthma symptoms. Furthermore, JPAC has an item for symptom of wheezing during exercise in last 1 month, it is considered not to miss the symptom of exercise-induced bronchoconstriction.

We also revealed that asthmatic children tended to have lower neopterin and biopterin levels. While serum neopterin level reflects the rate of BH₄ production, serum biopterin level directly reflects systemic pool of BH₄. Insufficiency of BH₄ during NO synthesis will cause NOS uncoupling, which induce superoxide generation.¹⁷ Therefore, asthma patients who do not have sufficient amount of biopterin are likely to have higher risk for exacerbation of asthma (Fig. 2). Additionally, analysis for JPAC Q4 revealed that wheezing during exercise was correlated with serum biopterin. This data indicated that higher biopterin level will reduce the frequency of wheezing during exercise.

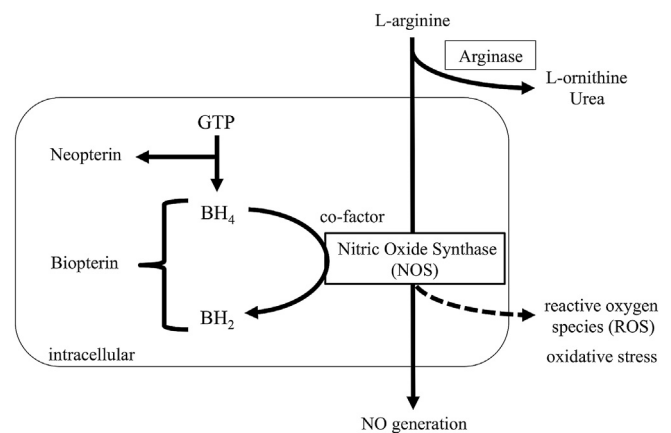


Fig. 2. BH₄ synthesis pathway and NO generation. BH₄ is biosynthesized from GTP and serves as a cofactor for NOS to produce NO from L-arginine. Inducible NOS (iNOS) produce FeNO by coupling with BH₄ oxidation to BH₂. Neopterin is intermittent metabolite of BH₄. Serum biopterin level directly reflects systemic pool of BH₄. Insufficiency of BH₄ during NO synthesis causes NOS uncoupling, which induces generation of reactive oxygen species (ROS). GTP, guanosine triphosphate; BH₄, tetrahydrobiopterin; BH₂, dihydrobiopterin.

In consistent with our findings, insufficiency of L-arginine, which is required for NO synthesis as substrate, has been demonstrated to develop asthma in animal models. Zhang *et al.* orally administered L-arginine to mice model of asthma and showed that L-arginine ameliorated airway hyperresponsiveness.²⁵ Similarly, Arikian-Ayyildiz *et al.* administered L-arginine by inhalation to a mice model of asthma and L-arginine reduced eosinophilia in BAL compared with placebo.²⁶ Inhibiting arginase can increase bioavailability of L-arginine and also have shown to reduce the airway sensitivity in guinea pigs.²⁷ Based on these results from the animal studies, clinical trials on asthma patients had been conducted.

There are several limitations need to be considered when applying our findings to clinical practice. Since we did not include normal individuals for this analysis, optimal threshold for diagnosis of asthma could not be estimated. Furthermore, currently biopterin cannot be measured in commercial laboratory. Fukushima and Nixon established the method to measure biopterin using fluorescence detection in the HPLC analysis.²⁸ It only requires antioxidant, such as ascorbic acid to prevent oxidization immediately after blood drawing. Therefore it can be measured with reasonable cost. Using their method, biopterin can be measured in serum, urine, cerebrospinal fluid, and even in exhaled breath condensate. In terms of reproducibility, Ogiwara *et al.* reported the SD of serum neopterin was 0.2–0.3 nmol/L, and same of biopterin was 0.4 nmol/L measured by HPLC.²⁹ However, further larger cohort studies are needed to determine the sensitivity and specificity of biopterin as an index of predicting asthma control.

In conclusion, we found that serum biopterin effect the following period's control status of asthmatic children. Assessing control status is essential for asthma management to adjust treatment. Further study is needed to optimize biomarker-guided management to improve the outcome of childhood asthma.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.alit.2018.08.012>.

Conflict of interest

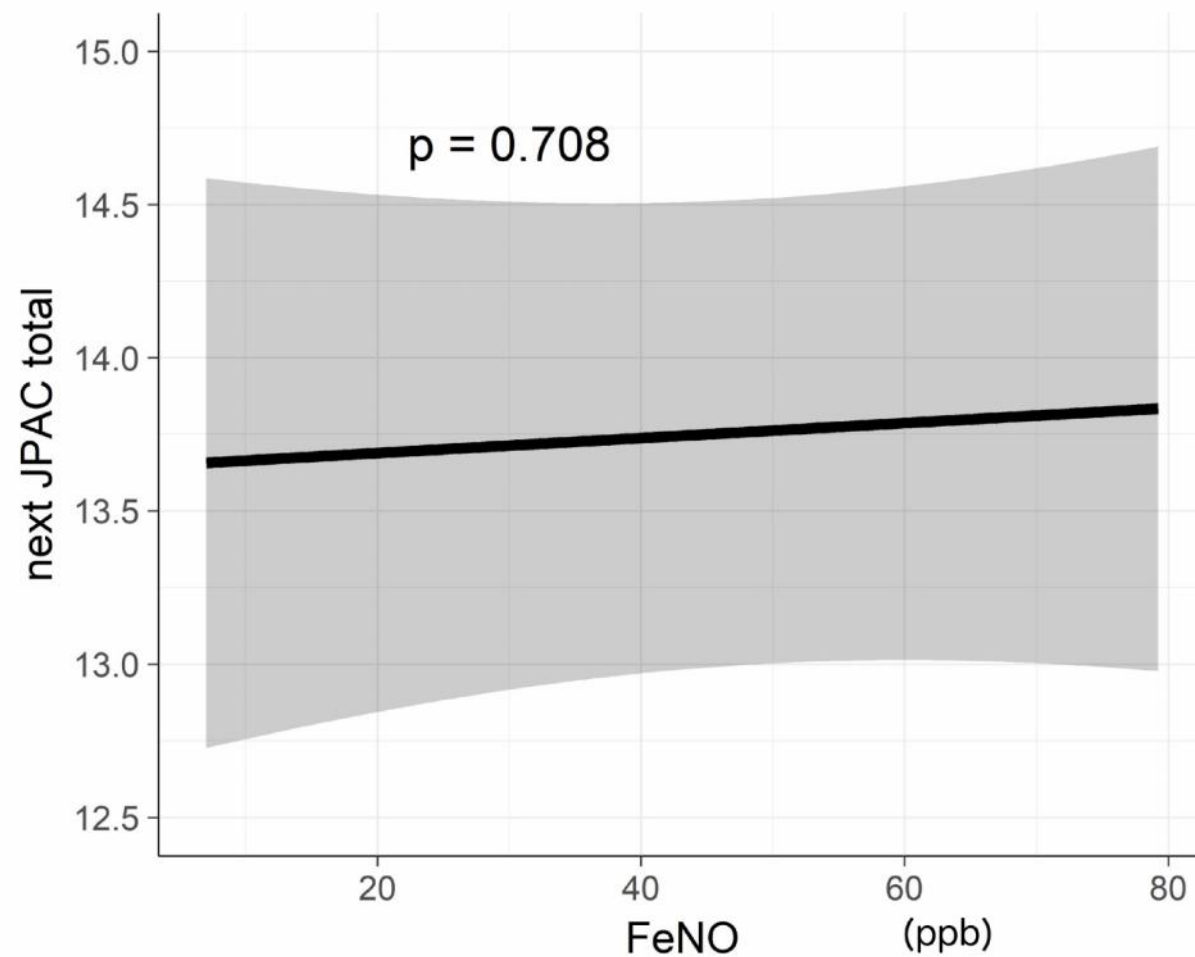
The authors have no conflict of interest to declare.

Authors' contributions

SKa designed and conducted the study and wrote the manuscript. TS and SKu carried out the biochemical analysis and quality assessments. SN, YH, SF and HF assisted patient recruitment and clinical assessment. DK and AS assisted in the design of the study and commented on the statistical analysis. TH and HS participated in the design and coordination and assisted with writing the manuscript. All authors read and approved the final manuscript.

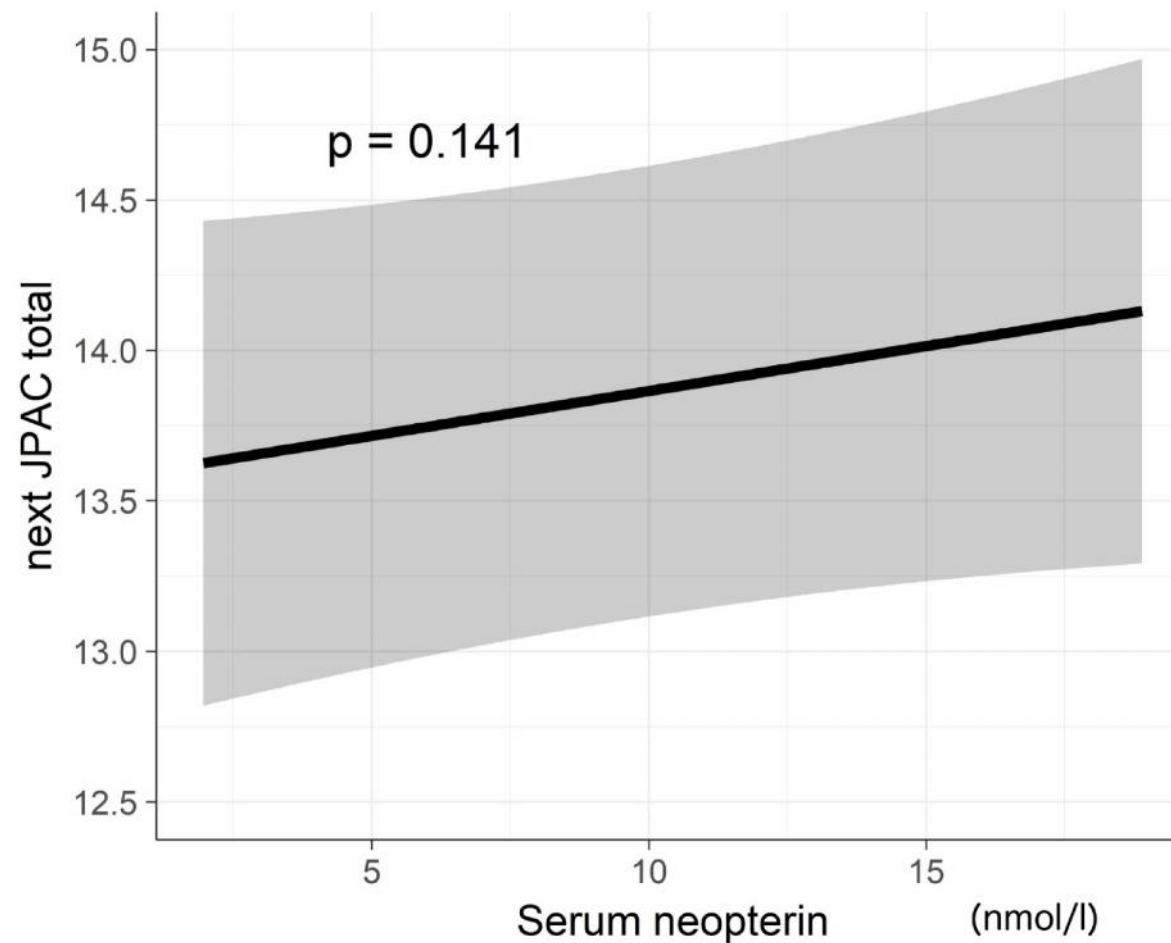
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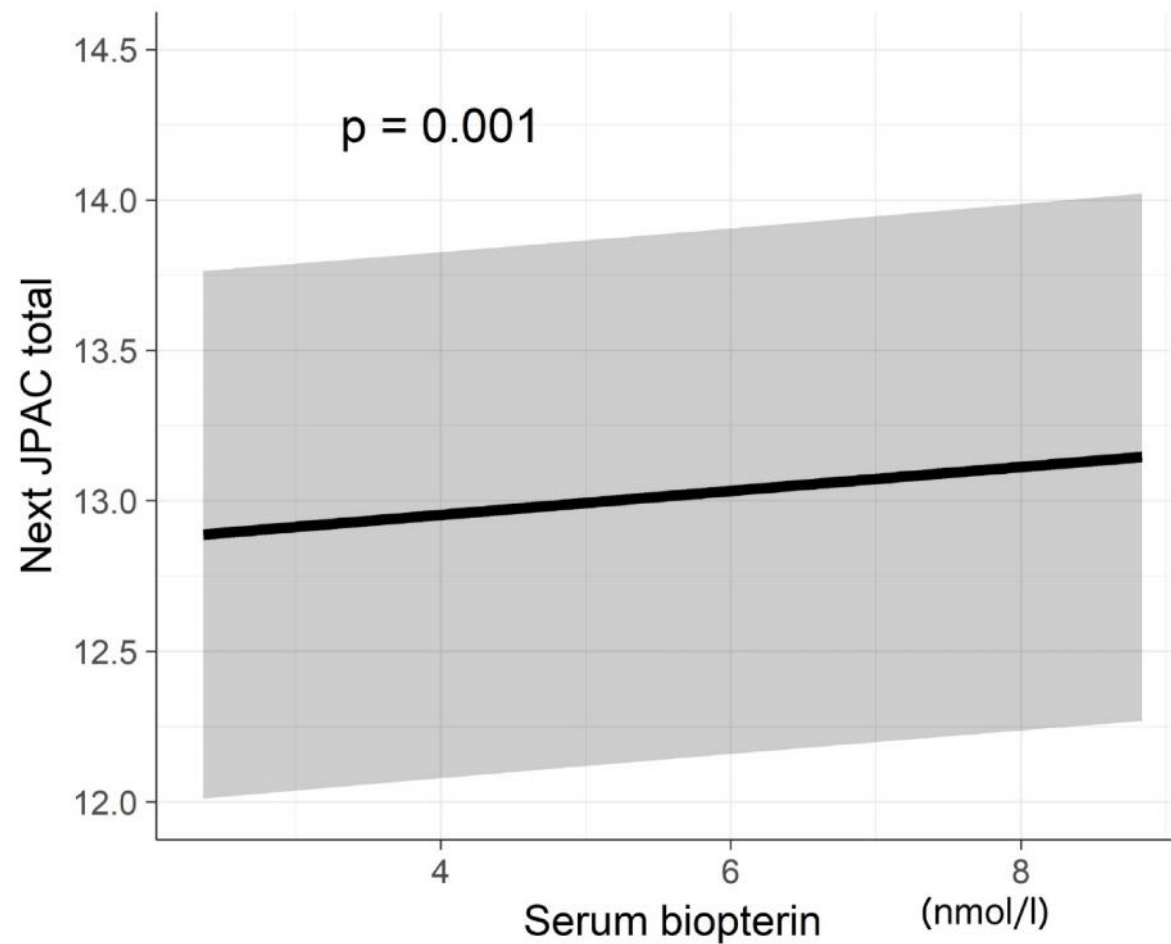
Supplementary Fig. 1. The impact of FeNO on JPAC score.

We assessed the impact of FeNO on JPAC score using multivariable linear regression analysis. JPAC: Japanese Pediatric Asthma Control Program, Asthma symptom control levels were defined using by JPAC total score (0-11: poor-controlled, 12-14: favorable-controlled, 15: complete-controlled). Next JPAC total: following period's JPAC total score. The gray shading part in figures showed 95% confidence interval (CI).



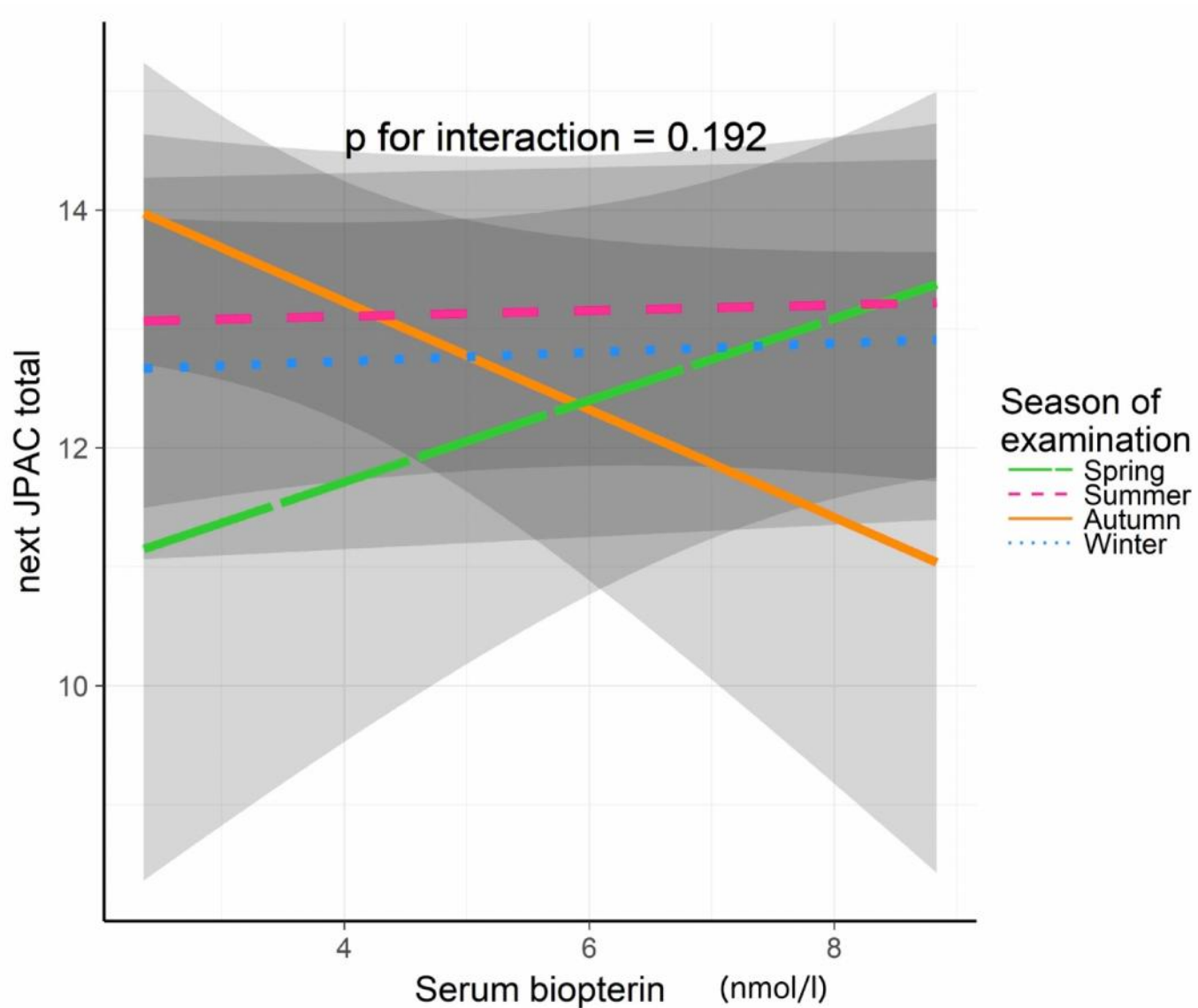
Supplementary Fig. 2. The impact of neopterin on JPAC score.

We assessed the impact of neopterin on JPAC score using multivariable linear regression analysis. JPAC: Japanese Pediatric Asthma Control Program, Asthma symptom control levels were defined using by JPAC total score (0-11: poor-controlled, 12-14: favorable-controlled, 15: complete-controlled). Next JPAC total: following period's JPAC total score. The gray shading part in figures showed 95% confidence interval (CI).



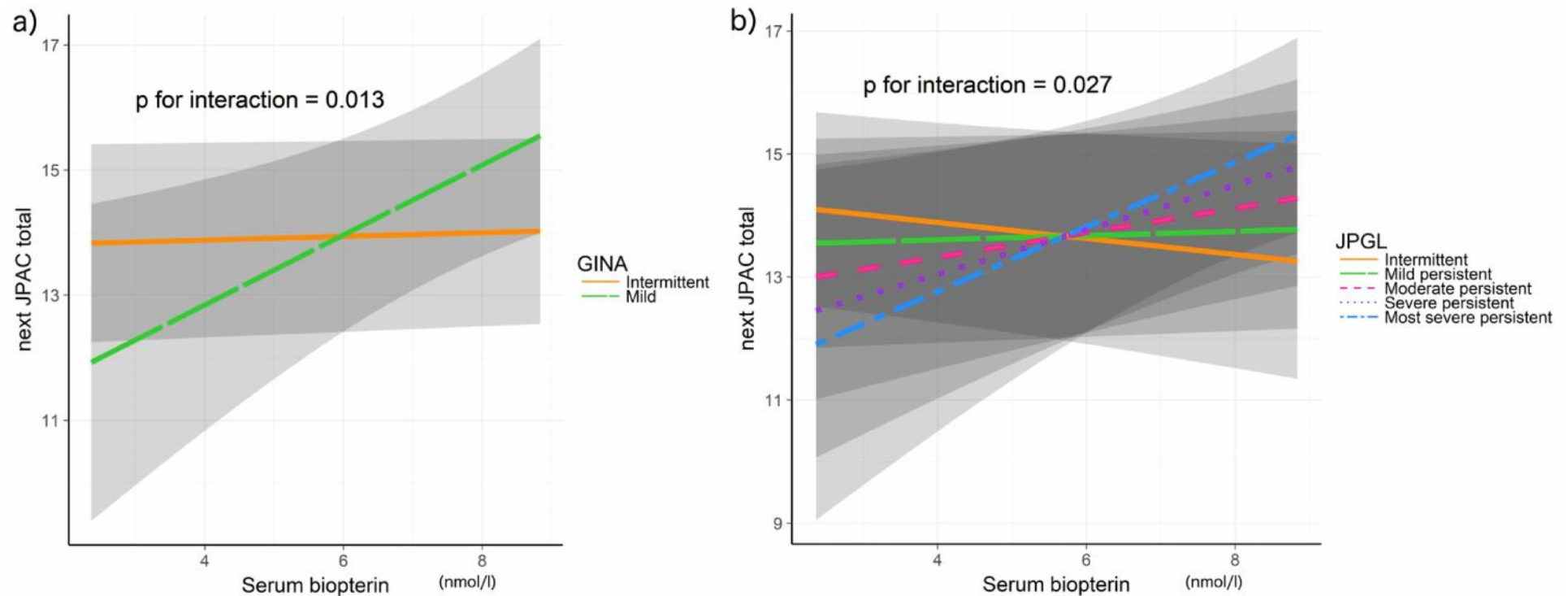
Supplementary Fig. 3. The impact of biopterin on JPAC score.

We assessed the impact of biopterin on JPAC score using multivariable linear regression analysis. JPAC: Japanese Pediatric Asthma Control Program, Asthma control levels were defined using by JPAC total score (0-11: poor-controlled, 12-14: favorable-controlled, 15: complete-controlled). Next JPAC total: following period's JPAC total score. The gray shading part in figures showed 95% confidence interval (CI).



Supplementary Fig. 4. The effect modification by seasons for biopterin.

Multivariable linear regression models were conducted with the cross-product term between biopterin and seasons. JPAC: Japanese Pediatric Asthma Control Program, Asthma symptom control levels were defined using by JPAC total score (0-11: poor-controlled, 12-14: favorable-controlled, 15: complete-controlled). The gray shading part in figures showed 95% confidence interval (CI).



Supplementary Fig. 5. The effect modification for biopterin by asthma severities.

Multivariable linear regression models were conducted with the cross-product term between biopterin and seasons. JPAC: Japanese Pediatric Asthma Control Program, Asthma symptom control levels were defined using by JPAC total score (0-11: poor-controlled, 12-14: favorable-controlled, 15: complete-controlled). The gray shading part in figures showed 95% confidence interval (CI). Multivariable linear regression models were conducted with the cross-product term between biopterin and the asthma severities.

(a) Severities were classified by GINA.

(b) Severities were classified by JPGL.

JPAC: Japanese Pediatric Asthma symptom Program, Asthma control levels were defined using by JPAC total score (0-11: poor-controlled, 12-14: favorable-controlled, 15: complete-controlled). The gray shading part in figures showed 95% confidence interval (CI).