

ORIGINAL ARTICLE

Asthma and Rhinitis

Prospective predictors of exacerbation status in severe asthma over a 3-year follow-up

H. Kimura¹ | S. Konno¹  | H. Makita¹ | N. Taniguchi¹ | K. Shimizu¹ | M. Suzuki¹ |
 H. Kimura¹ | H. Goudarzi¹ | Y. Nakamaru² | J. Ono³ | S. Ohta⁴ | K. Izuhara⁴ |
 Y. M. Ito⁵ | S. E. Wenzel⁶ | M. Nishimura¹ | for the Hi-CARAT investigators

¹Department of Respiratory Medicine, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Sapporo, Japan

²Otolaryngology Head and Neck Surgery, Hokkaido University School of Medicine, Sapporo, Japan

³Shino-Test Corporation, Kanagawa, Japan

⁴Division of Medical Biochemistry, Department of Biomolecular Sciences, Saga Medical School, Saga, Japan

⁵Department of Biostatistics, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Sapporo, Japan

⁶University of Pittsburgh Asthma Institute at UPMC/University of Pittsburgh School of Medicine, Pittsburgh, PA, USA

Correspondence

Satoshi Konno, Department of Respiratory Medicine, Faculty of Medicine and Graduate School of Medicine, Hokkaido University, Sapporo, Japan.
 Email: satkonno@med.hokudai.ac.jp

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Summary

Background: A predisposition to exacerbations is being recognized as a distinct phenotype with “previous exacerbations” representing the strongest clinical factor associated with future exacerbation. Thus, to identify additional novel biomarkers associated with asthma exacerbations, “past exacerbation status” must be included as a confounding factor.

Objective: This study aimed to characterize the clinical and biomarker features associated with asthma exacerbations in severe asthma.

Methods: We evaluated clinical parameters from 105 severe asthmatics yearly for 3 years, as well as their exacerbation status. We classified the subjects into 3 groups: (i) consistent non-exacerbators (CNE, subjects who did not experience any exacerbation over the 3-year period); (ii) consistent frequent exacerbators (CFE, subjects with frequent exacerbation, defined as those who had 2 or more exacerbations within 1 year, throughout the 3-year period); and (iii) intermittent exacerbators (IE). We conducted multivariate analysis for comparisons among the groups for multiple factors, including several Th2-related biomarkers, in addition to the “past exacerbation status.”

Results: Thirty-nine subjects were classified as CNE, 15 as CFE, and 51 as IE. Frequent exacerbations in the previous year predicted exacerbations for the following year ($P < .001$). Among the several Th2-related biomarkers, only FeNO was associated with exacerbation status. When we analysed the data after the second visit, the impact of FeNO on predicting future exacerbation remained significant, even after considering the exacerbation status during the first year ($P < .05$).

Conclusions and Clinical Relevance: Measurement of FeNO has a significant potential to predict future asthma exacerbation, which is independent of the “past exacerbation history.”

KEYWORDS

asthma, biomarker, eosinophils, exacerbation, FeNO, pneumology

1 | INTRODUCTION

Asthma is recognized as a heterogeneous and complex disease, with distinct therapeutic approaches which depend upon its phenotypic classification.^{1–3} “Exacerbation-prone” asthma has been identified as a potentially distinct asthma phenotype, linked with high morbidity and mortality in people with asthma.^{4–8} In fact, prevention of asthma exacerbations is one of the major goals of current asthma treatment guidelines.⁹ Therefore, identifying patients at the greatest risk of future exacerbations and achieving good asthma control is key to preventing future exacerbations and ultimately reducing health care costs.

With regard to the clinical predictive factors for future asthma exacerbation, the significance of the so-called “T-helper 2 (Th2)-related inflammatory biomarkers,” including blood/sputum eosinophil levels, immunoglobulin E level (IgE), and fractional excretion of nitric oxide (FeNO) concentration, has been investigated in many retrospective studies.^{4,5,10,11} Meanwhile, “past exacerbation history” has been identified as the strongest factor with which to predict future asthma exacerbation.^{7,8} Despite the existence of this simple and readily-obtainable clinically information, no studies have considered this factor in relation to the significance of biomarkers. In other words, to identify any novel biomarkers with clinical utility, we should evaluate whether any biomarkers could be independent predictors of the “future asthma exacerbations,” over that of exacerbation history alone. Most previous studies have been retrospective or with limited 1-year follow-up.^{4,5,7–12}

In this study, using the obtained at the subjects’ study entry point for the Hokkaido Severe Asthma Cohort Study, we previously reported the clinical characteristics of such subjects, with particular focus upon upper and lower airway inflammation and airflow limitation in relation to smoking status.¹³ We recently finished the 3-year follow-up, in which we yearly made repeated measurements of clinical and biological parameters.

The goal of the current study was to characterize the clinical and biomarker features associated with asthma exacerbations in severe asthma. In particular, we aimed to evaluate the clinical utility of several Th2-related biomarkers for the prediction of future asthma exacerbation over that of exacerbation history alone.

2 | MATERIALS AND METHODS

The details of the materials and methods used in this study were described in our previous report¹³ and can be found in the Supporting Information.

2.1 | Study protocol and subjects

The Hokkaido Severe Asthma Cohort Study (the Hokkaido-based Investigative Cohort Analysis for Refractory Asthma [Hi-CARAT]) is a multicenter observational cohort study, which primarily aims

to characterize subjects with severe asthma, including those who smoke. The details of this study protocol have been reported previously.¹³ Patients with severe asthma were enrolled at Hokkaido University Hospital and its 29 affiliated hospitals and clinics between February 2010 and September 2012. The diagnosis of severe asthma was based on the American Thoracic Society (ATS) criteria for refractory asthma published in 2000,¹⁴ with slight modification. In brief, we also included the criteria as follows. The diagnosis of severe asthma required one or both major criteria and 2 minor criteria. Patients who were well-controlled under the current medications were asked if within 1 year they experienced episodic deterioration of symptoms, urgent care visits, and rescue use of short-acting bronchodilators when the dose of the current medication was reduced.¹³

A total of 127 patients with severe asthma were selected for baseline analyses.¹³ A flow chart demonstrating the study process from the initial screening of Visit Entry (VE) to Visit 1-year (V1), Visit 2-year (V2), and the end of the 3-year follow-up period (Visit 3-year, V3) is depicted in Figure S1.

2.2 | Assessment of exacerbation

We defined asthma exacerbation based on the need for systemic corticosteroids for more than 3 days and/or hospital admission, which corresponds to “severe exacerbation” based on the European Respiratory Society (ERS)/ATS guideline of severe asthma.¹⁵ Frequent exacerbators were defined as those who had 2 or more exacerbations within 1 year of follow-up, whereas non-exacerbators were defined as those who did not have any annual events.

2.3 | Measurement of biomarkers

The concentration of FeNO was measured with the NIOX MINO® (Aerocrine, Stockholm, Sweden) using a single-breath online method, in accordance with the ATS guidelines.¹⁶ We also measured blood eosinophil counts, serum total/specific IgE, and serum periostin level. Detail methods of measurements of other biomarkers were described in our previous report¹³ and can be found in the Supporting Information.

2.4 | Questionnaires

Upon study entry and during every hospital visit, the clinical research coordinators (CRCs) directly administered comprehensive questionnaires to all subjects; the information assessed included their asthma symptom score (as assessed by the asthma control test), as well as symptoms of gastroesophageal reflux disease (GERD), depression, and allergic rhinitis (see Supporting Information). The presence of aspirin-exacerbated respiratory disease (AERD) was diagnosed by the chief respiratory physician and confirmed by the CRCs.

2.5 | Pulmonary function testing

Spirometry was performed before and after inhalation of 400- μ g oxitropium and 400- μ g salbutamol. Further details of the pulmonary function tests are described in our previous report.¹³

2.6 | Assessment of adherence to medications

As described in our previous study,¹³ we excluded subjects who were assessed by a doctor (Dr. NT) and the CRCs to have poor adherence to their medications and inadequate medication inhalation technique upon their initial visit. Table S1 shows the patients' adherence to their medications during the study period. Subjects with low adherence (<70%) were excluded from the final analyses.

2.7 | Statistical analysis (Figure 1)

2.7.1 | Analysis 1 (from VE to V3: 3-year follow-up)

To assess the stability of the frequent exacerbation phenotype over the 3-year follow-up period, we classified subjects into 3 groups: (i) consistent non-exacerbators (CNE), subjects who did not have any exacerbation episodes over 3 years; (ii) consistent frequent exacerbators (CFE), subjects who fulfilled the criteria for frequent exacerbation throughout the entire 3-year period; and (iii) intermittent exacerbators (IE), subjects who did not fulfil either of the 2 previously described criteria. Appropriate methods of statistical analyses were used (see Supporting Information). In the multivariate

analyses, we considered age, sex, body mass index (BMI), atopic status, smoking status (pack year [PY] $\geq 10^{13}$), asthma duration, use of oral corticosteroids (OCS), and the presence of AERD (Model 1, Figure 1).

2.7.2 | Analysis 2 (from V1 to V3: 2-year follow-up)

To confirm the results from Analysis 1 (from VE to V3), using the data at initial entry (VE), we conducted similar analyses using the data obtained at V1, over 2-year follow-up (from V1 to V3; Figure 1). We classified subjects into 3 groups: (i) consistent non-exacerbators in analysis 2 (CNE-2), subjects who did not have any exacerbation episodes over 2 years; (ii) consistent frequent exacerbators in analysis 2 (CFE-2), subjects who fulfilled the criteria for frequent exacerbation throughout the 2-year period; and (iii) intermittent exacerbators in analysis 2 (IE-2), subjects who did not fulfil either of the 2 previously described criteria. In the multivariate analyses, we also considered the "exacerbation status during the first year" (Model 2, Figure 1). In addition to generalized estimating equations (GEE), models for repeated binary outcomes were applied to calculate odds ratios for the associations of several variables with repeated measures of exacerbation status.

Statistical analyses were performed using the statistical software package SYSTAT for Windows, version 13.1 (SYSTAT, San Jose, CA, USA), EZR, version 1.27 (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for the R software (The R Foundation for Statistical Computing, Vienna,

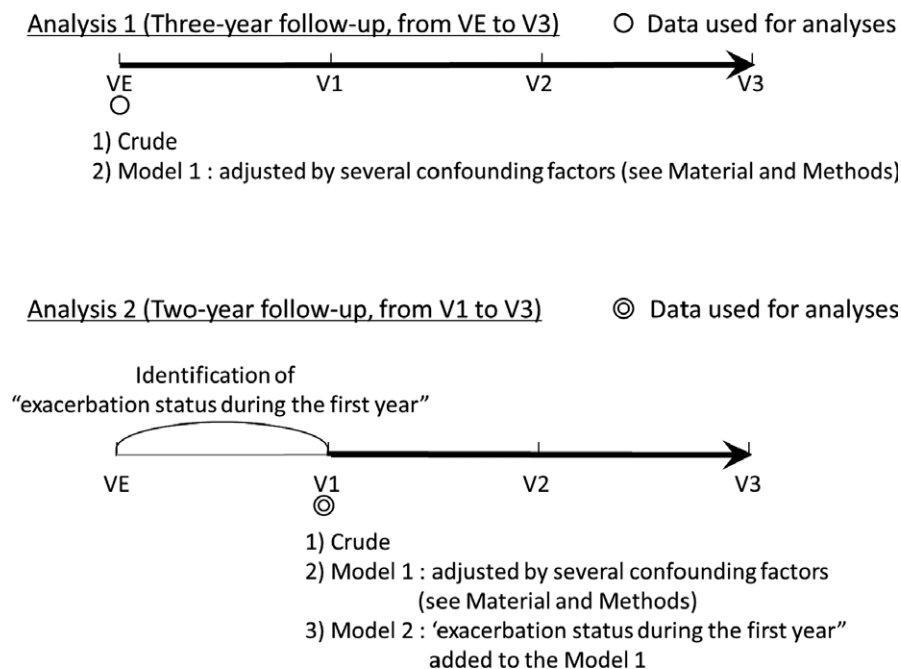


FIGURE 1 The study protocol of this study. Once a year, all subjects visited Hokkaido University and underwent serial clinical evaluations after confirming that their asthmatic symptoms were unchanged during the past 2 wk. Analysis 1: Analyses over 3-y follow-up using the data obtained at VE. Analysis 2: Analyses over 2-y follow-up using the data obtained at V1. Abbreviations: V1, Visit 1-y; V2, Visit 2-y; V3, Visit 3-y; VE, Visit entry

Austria),¹⁷ and SAS University edition (SAS institute, Cary, NC, USA). For all statistical analyses, $P < .05$ was considered significant.

3 | RESULTS

3.1 | Study protocol

A flow chart is shown in Figure S1. Once a year, all subjects visited Hokkaido University and underwent serial clinical evaluations after confirming that their asthmatic symptoms were unchanged during the past 2 weeks. During the 3-year follow-up period, 10 subjects were lost to follow-up due to withdrawal of consent, 5 subjects were excluded due to unavailability of exacerbation data, 2 subjects were excluded due to poor adherence to their medications, and 5 subjects were excluded due to unavailability of their adherence status. Additionally, 3 subjects missed Visit 1-year (V1) to Hokkaido University and were also excluded. The subjects who missed Visits 2-year (V2) and 3-year (V3), but continued to record in their asthma diaries, as well as those who visited the chief doctors in the hospital/clinic, were included in the final analysis. Ultimately, a total of 105 subjects were analysed in the current study.

3.2 | Subject demographics

Table 1 reports the demographics of the subjects who were analysed in this study. At the time of enrolment, the mean age was 58.5 ± 12.1 years (range, 29–83 years) and the mean duration of

asthma was 19.7 ± 14.6 years (range, 1–61 years); 42.9% of the patients were male ($n = 45$) and 61.9% were atopic ($n = 65$). Eleven (10.5%) subjects were current smokers, and 56 (53.3%) were ex-smokers. Thirty-nine (37.1%) subjects used daily OCS.

3.3 | Number of exacerbations over the 3-year follow-up period (Analysis 1: from VE to V3)

Over the 3-year study period, 64 subjects (63%) had at least 1 exacerbation. As shown in Figure S2, the number of exacerbations that occurred in each subject varied widely, from 0 to 43. The median number of exacerbations per patient over the 3-year period was 1 (interquartile range [IQR], 0–4).

3.4 | Stability of the exacerbation status over the 3-year study period

As shown in Figure 2, among the 54.3% of subjects who were classified as non-exacerbators during the first year, 80.7% of the subjects remained free from exacerbations during the second year of the study. Among the 30.5% of subjects who were classified as frequent exacerbators during the first year of the study, 59.4% continued to have frequent exacerbations during the second year as well. Additionally, 84.8% and 78.9% of those classified as non-exacerbators and frequent exacerbators, respectively, in both the first and second years remained the same during the third year. Over the 3 years of follow-up, 39 subjects (37.1%) were classified as consistent non-

TABLE 1 Characteristics of the subjects at study entry according to the exacerbation status groups in 3-y follow-up (Analysis 1)

	Type of exacerbation				P-value	P for trend*
	All (N = 105)	CNE (N = 39)	IE (N = 51)	CFE (N = 15)		
Male sex, N (%)	45 (42.9)	14 (35.9)	26 (51.0)	5 (33.3)	.259	n/a
Age at enrolment, y	58.5 ± 12.1	57.3 ± 11.8	60.0 ± 12.2	56.3	.456	n/a
Asthma duration, y	19.7 ± 14.6	16.8 ± 11.1	22.0 ± 16.5	19.3 ± 15.4	.242	n/a
Smoking status (Current/Ex/Never)	11/56/38	4/17/18	7/29/15	0/10/5	.272	n/a
Pack years	5.5 (0–23.4)	4.5 (0–17.1)	7.4 (0–30.9)	4.0 (0–11.6)	.237	n/a
Pack years ≥ 10 , N (%)	46 (43.8)	14 (35.9)	25 (49.0)	7 (46.7)	.448	n/a
Body mass index, kg/m ²	25.5 ± 5.0	25.7 ± 5.9	25.5 ± 3.9	24.7 ± 5.7	.795	n/a
Daily ICS dose, μg^a	1638 ± 518.8	1674.4 ± 462.7	1611.3 ± 455.4	1640 ± 819.2	.852	n/a
Maintenance OCS use, N (%)	39 (37.1)	13 (33.3)	17 (33.3)	9 (60.0)	.141	n/a
Atopy, N (%)	65 (61.9)	26 (66.7)	30 (58.8)	9 (60.0)	.740	n/a
ACT	21.0 (17.0–23.0)	22.0 (18.3–23.8)	20.0 (16.3–23.0)	20.0 (15.3–20.8)	.107	.039
AQLQ	5.5 (4.9–6.3)	5.7 (4.9–6.3)	5.5 (4.9–6.3)	5.1 (4.3–6.1)	.341	.160

ACT, asthma control test; AQLQ, asthma quality of life questionnaire; CFE, consistent frequent exacerbators; CNE, consistent non-exacerbators; ICS, inhaled corticosteroids; IE, intermittent exacerbators; n/a, not applicable; OCS, oral corticosteroids.

Data are shown as the mean $\pm$ standard deviation, median (interquartile range), or number (%).

P-values were obtained using a chi-square test, 1-way analysis of variance (ANOVA), or the Mann–Whitney *U* test, as appropriate.

^aEquivalent to budesonide dose.

*Jonckheere–Terpstra trend test.

exacerbators (CNE), 15 subjects (14.3%) as consistent frequent exacerbators (CFE), and 51 (48.6%) as intermittent exacerbators (IE; Table 1). Statistically, the exacerbation status during the previous year was most significantly associated with the exacerbation status during the next year of follow-up ($P < .0001$; Tables S2 and S3).

3.5 | Correlations between blood/sputum biomarkers

As shown in Figure S3, blood eosinophil counts and serum periostin (A), blood eosinophil counts and FeNO (B), FeNO and serum periostin (C), and total IgE and serum periostin (D), obtained at the entry, were correlated with each other ($P < .05$). However, their correlations were not very strong ($R = .35-.5$, $P < .01$), suggesting both similar and unique contributions of each parameter.

3.6 | Baseline clinical characteristics according to exacerbation status

Tables 1 and 2 show the clinical characteristics at baseline (VE) when the subjects were classified according to their exacerbation status. There were no significant differences in age, age of onset, BMI, and smoking status. The pulmonary function test results, including %FEV₁, FEV₁/FVC, and reversibility, were also not significantly different among the 3 groups. The asthma symptom score was lowest in the CFE group (P for trend = .039), but this trend was not observed when the Asthma Quality of Life Questionnaire (AQLQ) was used for assessment instead of the asthma control test. The scores for GERD (F-scale) and depression (Self-rating Depression Scale, SDS) did not differ among the 3 groups. The presence of AERD tended to be higher in the CFE group (P for trend = .069).

Among the several Th2-related biomarkers, blood eosinophil count and FeNO concentration were significantly higher in the CFE group than in the other 2 groups (P for trend = .032 and 0.013, respectively; Table 2 and Figure 3). However, total serum IgE and serum periostin levels did not differ between the 3 groups. The presence of allergic rhinitis and nasal polyps and the sinus score, as assessed with computed tomography, were not associated with the exacerbation status (Table 2). The multivariate analysis revealed that FeNO concentration was an independent factor associated with CFE ($P = .013$), and the blood eosinophil demonstrated a tendency ($P = .063$; Table 3). Figure S4 shows the Kaplan–Meier curve demonstrating the number of days until the first asthma exacerbation over the 3-year study period. Results from the Cox proportional hazards model revealed that only FeNO concentration was significantly associated with the first exacerbation (hazards ratio [HR]: 2.42, 95% confidence interval [CI]: 1.16–5.04, $P = .018$; Table S4).

3.7 | Clinical evaluation after 1 year (Analysis 2: from V1 to V3)

To confirm the results from Analysis 1 (from VE to V3), using the data at initial entry (VE), we conducted similar analyses using the

data obtained at V1, over 2-year follow-up (from V1 to V3; Figure 1). The detailed clinical characteristics of the subjects obtained at V1 are reported in Tables S5 and S6. Clinical evaluations were completed for 102 subjects at V1. Among the 62 non-exacerbators assessed during the second year (from V1 to V2), 49 (79.0%) remained free from exacerbations during the third year (from V2 to V3). Among the 28 frequent exacerbators assessed during the second year, 19 (67.9%) had persistently frequent exacerbations in the third year (Figure S5). We classified the subjects into 3 groups according to their exacerbation status over a 2-year follow-up period after V1: (i) CNE-2, those who did not have any exacerbations for 2 years; (ii) CFE-2, those who had frequent exacerbations for 2 consecutive years; and (iii) IE-2, those who did not fulfil any of the other 2 criteria.

Over the 2-year follow-up, 49 subjects were classified as CNE-2 (48.0%), and 19 as CFE-2 (18.6%). When we compared the characteristics of the 3 groups using the data obtained during V1, the FeNO and the presence of AERD were significantly different between the 3 groups (P for trend = .0027, and .021, respectively). The blood eosinophil level did not show statistical significance (P for trend = .463; Table S6). The impact of FeNO concentration on exacerbation status over the 2-year follow-up remained significant after adjustment of several factors (Model 1, $P = .007$; Table S7). Of importance, the impact of FeNO remained significant even after adjustment of the exacerbation status during the first year (Model 2, $P = .009$; Table S7; $P = .029$; Table S8, $P = .003$; Table S9).

4 | DISCUSSION

In this study, our intention was to clarify the clinical characteristics and determinants of severe exacerbations in severe asthmatic subjects enrolled in the Hokkaido Severe Asthma Cohort Study. Our 3-year follow-up of severe asthmatic subjects, which included repeated measurements of a range of clinical parameters, allowed us to confirm that asthma exacerbation was a stable phenotype. In addition, we evaluated the clinical utility of several Th2-related biomarkers for their ability to predict future exacerbation status. Notably, multivariate analyses demonstrated that the impact of FeNO upon the prediction of future asthma exacerbations remained significant even when considering “past exacerbation status” (Table 3). These observations suggest the potential utility of FeNO to help in identifying risk for exacerbation in addition to “past exacerbation status.”

The identification of novel biomarkers and their clinical utility with regard to the future risk of exacerbation must be evaluated in light of the known strong influence of “past exacerbation status.” In the case of COPD, Hurst et al¹⁸ attempted to identify factors which could predict future exacerbation. In their univariate analysis, several blood biomarkers were identified as potential predictive factors of future exacerbation. However, in their multivariate analysis, all of these significant associations disappeared when considering the “past exacerbation history,” and the authors finally concluded that the information relating to “past exacerbation status” might be

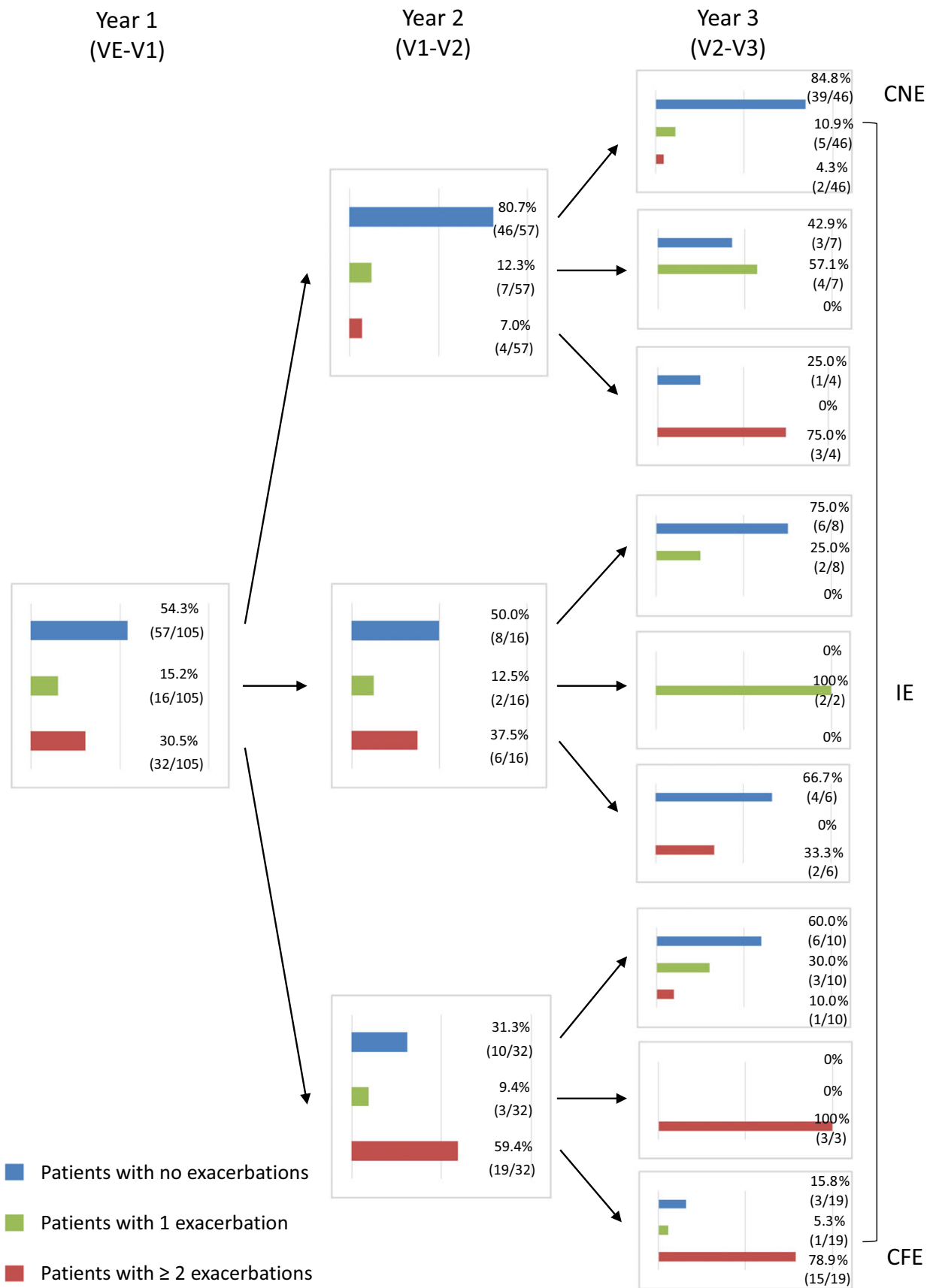


FIGURE 2 Stability of the exacerbation frequency status over the 3-y follow-up period (Analysis 1). Abbreviations: V1, Visit 1-y; V2, Visit 2-y; V3, Visit 3-y; VE, Visit entry

TABLE 2 Comorbidity characteristics of the subjects at study entry according to the exacerbation status groups in 3-y follow-up (Analysis 1)

		Type of exacerbation				
	All (N = 105)	CNE (N = 39)	IE (N = 51)	CFE (N = 15)	P-value	P for trend*
Biomarkers						
Blood eosinophil, cells/ μ L	197.0 (0.52)	149.7 (0.52)	211.0 (0.52)	318.1 (0.45)	.095	.032
Blood neutrophil, cells/ μ L	4528.2 (0.18)	4453.2 (0.19)	4568.3 (0.16)	4589.6 (0.23)	.952	.862
Total serum IgE, IU/mL	138.5 (0.70)	163.9 (0.74)	127.4 (0.70)	118.8 (0.60)	.709	.301
Sputum eosinophil, % ^a	8.0 (0.8-30.6)	5.1 (0.7-26.3)	4.8 (0.8-31.1)	20.0 (9.2-41.6)	.349	.670
FeNO, ppb	30.2 (0.36)	23.1 (0.35)	34.3 (0.34)	39.0 (0.39)	.033	.013
Serum periostin, ng/mL	80.3 (0.21)	77.4 (0.22)	82.8 (0.20)	79.3 (0.21)	.806	.462
Pulmonary function						
FEV ₁ , % predicted (maximum) ^b	91.4 $\pm$ 18.9	93.7 $\pm$ 18.0	89.9 $\pm$ 19.7	90.5 $\pm$ 19.5	.627	.529
FEV ₁ /FVC, % ^c	66.3 $\pm$ 12.7	67.9 $\pm$ 12.3	64.9 $\pm$ 12.8	67.1 $\pm$ 13.8	.526	.599
Co-morbidity						
AERD (%)	15 (14.3)	4 (10.3)	6 (11.8)	5 (33.3)	.073	.069
Allergic rhinitis (%)	85 (81.0)	32 (82.1)	40 (78.4)	13 (86.7)	.756	.875
COPD ^d (%)	64 (61.0)	23 (59.0)	31 (60.8)	10 (66.7)	.873	.632
LMS ^e	3.0 (0-9.3)	3.0 (0-8.0)	4.0 (1.0-12.0)	4.0 (1.3-7.5)	.446	.427
Nasal polyp ^e , N (%)	28 (26.7)	9 (23.1)	15 (29.4)	4 (26.7)	.797	.649
GERD, F-scale	8.0 (3.0-13.3)	7.0 (2.0-13.0)	8.0 (3.0-13.8)	10.0 (6.3-13.8)	.758	.488
SDS	38.0 (32.0-46.0)	37.0 (31.0-46.0)	39.0 (33.0-45.0)	39.0 (31.3-45.8)	.683	.484

AERD, aspirin-exacerbated respiratory disease; CFE, consistent frequent exacerbators; CNE, consistent non-exacerbators; FeNO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in 1 s; FVC, forced vital capacity; GERD, gastroesophageal reflux disease; IE, intermittent exacerbators; LMS, Lund-Mackay score; SDS, self-reported depression scale.

Data are shown as the mean $\pm$ standard deviation (SD), median (interquartile range), geometric mean (log₁₀ SD), or number (%).

P-values were obtained using a chi-square test, 1-way analysis of variance (ANOVA), or the Mann-Whitney U-test, as appropriate.

^aN = 96 (NE, 33; IE, 49; FE, 14).

^bMaximum value of FEV₁ among the 4 procedures (see Methods).

^cFEV₁/FVC was applied with the value corresponding to the maximum FEV₁.

^dDefined as FEV₁/FVC <70%.

^eDetected by computed tomography.

*Jonckheere-Terpstra trend test or Cochran-Armitage trend test.

sufficient for the prediction of future exacerbation. In asthma studies, a number of molecules have been evaluated as potential novel biomarkers for the prediction of future exacerbation. However, none of the existing studies have concomitantly considered the strongest factor, the "past exacerbation status," when evaluating their clinical utility. With this regard, our multivariate analysis strongly supports the clinical utility of FeNO for the prediction of future exacerbation in addition to information on "past exacerbation status."

The importance of several potential biomarkers related to Th2-driven inflammatory pathways, such as blood/sputum eosinophil levels, total serum IgE level, FeNO concentration, and serum periostin level, has been extensively investigated in the management of asthma.^{4,5,10,11,13,19,20} In addition to their similarities, the unique contributions of these markers to the several asthma phenotypes have been previously discussed.^{13,19,21} In this study, we demonstrated that all these biomarker parameters correlated with each other. However, the correlations were modest ($R = .3-.5$), confirming the differential characteristics of these parameters. Among the

biomarkers, only FeNO concentration was consistently and most significantly associated with exacerbation status over the 3-year study period. The sputum eosinophil level tended to be associated with exacerbation status, suggesting that FeNO concentration is the parameter that would most accurately reflect airway inflammation which predisposes to exacerbations. Alternatively, several reports have suggested the distinct role of FeNO concentration in determining specific asthma phenotypes.²²⁻²⁴ Regardless of the mechanisms involved, our results suggest that FeNO concentration, which is a non-invasive and easily measurable marker, would be useful to predict future exacerbation risk in patients with severe asthma.

In contrast, serum periostin, which is an inducible extracellular matrix protein produced by IL-13 and also a potential biomarker of airway eosinophils,^{13,19,20} was not associated with exacerbations in our study. Recent studies have described the potential role of this molecule in facilitating airway remodelling and the potential serum markers that could predict a decline in pulmonary function in patients with asthma of varying severity.¹⁹

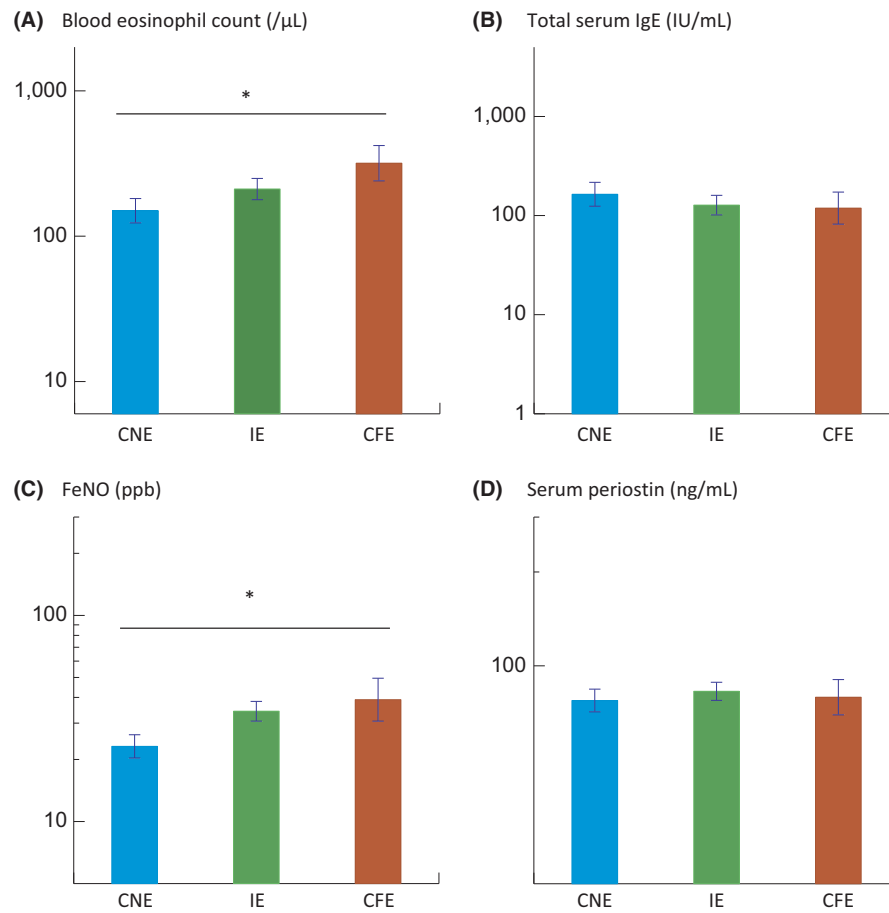


FIGURE 3 Comparison of the baseline characteristics according to exacerbation status (Analysis 1). A, Blood eosinophil (/μL). B, Total serum IgE level (IU/mL). C, FeNO (ppb). D, Serum periostin level (ng/mL). Abbreviations: CFE, consistent frequent exacerbators; CNE, consistent non-exacerbators; IE, intermittent exacerbators

TABLE 3 Comparison of the blood eosinophil count and FeNO among exacerbation status groups in 3-y follow-up (Analysis 1)

	Type of exacerbation			P-value crude	P-value adjusted*
	CNE (N = 39)	IE (N = 51)	CFE (N = 15)		
Blood eosinophil, cells/μL ^a	149.7 (0.52)	211.0 (0.52)	318.1 (0.45)	.095	.063
FeNO, ppb ^a	23.1 (0.35)	34.3 (0.34)	39.0 (0.39)	.033	.013

CFE, consistent frequent exacerbators; CNE, consistent non-exacerbators; FeNO, fractional exhaled nitric oxide; IE, intermittent exacerbators.

Data are shown as the geometric mean ($\log_{10}$ SD).

P-values were obtained using a 1-way analysis of variance (ANOVA).

^aLog-transformed.

*Model 1 (see Figure 1) Adjusted for age, asthma duration, sex, body mass index, atopic status, smoking status (PY $\geq$ 10), oral corticosteroid use, and aspirin-exacerbated respiratory disease.

The current asthma guidelines emphasize 3 major goals of asthma management: (i) relief of symptoms, (ii) reduction of future risk of exacerbation, and (iii) reduction in the decline of pulmonary function.⁷ Accordingly, measurement of the multiple biomarkers, including FeNO concentration, can improve the quality of asthma management, particularly in regard to reducing future risks. Due to the variable nature of pulmonary function, particularly in patients with severe asthma, we believe that a 3-year follow-up period was too short to identify the factors associated with change in

pulmonary function. As such, our study is still ongoing to reach a maximum of 6 years of follow-up in order to clarify the factors associated with the long-term clinical course of asthma.

In contrast to previous reports,^{4,25} the presence of several asthma comorbidities, such as chronic sinusitis, obesity, and GERD, was not associated with exacerbation status in our study. However, AERD was more frequently observed in the CFE group than the other 2 groups, which confirmed a previous retrospective study conducted in Japan.²⁶ Although the exact reasons are unclear, the

ethnic differences between the study's samples, ie, lower prevalence of obesity and/or GERD,²⁷ could explain for these discrepancies.

This study found that baseline pulmonary function, which was previously reported to be a significant factor associated with asthma exacerbation, also did not differ among the 3 groups. This may be due to the pulmonary function tests being done without a full withhold of asthma medications, perhaps levelling the measures. However, whether pulmonary function testing is done with or without a proper withhold of asthma medications could influence its utility in informing clinicians of exacerbation risk.

Our cohort studies were designed to clarify the various effects of smoking on asthma phenotypes. To achieve this goal, we intentionally included subjects who smoke/smoked and those with the potential coexistence of chronic obstructive pulmonary disease (COPD), who would require high doses of inhaled corticosteroids (ICS) and/or OCS for asthma control. We have shown that the degree of airway inflammation, which was assessed by measuring the blood and sputum eosinophils, as well as FeNO concentration, varied widely regardless of smoking status.¹³ Accordingly, our inclusion criteria of smokers with/without COPD for asthmatic subjects might have significantly affected the asthma outcomes.

In contrast to our current results, several previous reports showed that FeNO might not be useful in the prediction of future exacerbation.^{4,12} The poor adherence of patients to prescribed treatment has been put forward as the predominant reason for a high rate of asthma exacerbation. A high FeNO concentration was reported to be a marker of poor adherence to medications in patients with severe asthma.²⁸ In our cohort, we only focused upon severe asthmatic subjects who had been managed by respiratory physicians. Most of our subjects adhered well to medicines and we also carefully excluded subjects with poor medication adherence. This might be one of the explanations for the discrepancy between our present study and earlier reports. In fact, over 30% of our patients were on systemic corticosteroids. High FeNO levels have been reported to be the best independent factor associated with daily systemic corticosteroids.²⁹ Thus, high FeNO in a well-treated, severe asthma population may indicate a more complicated immune process, in general.

This study had some limitations. First, the sample size may have been too small, despite recruitment of subjects from 29 affiliated hospitals/pulmonary clinics. However, all subjects were carefully followed, and the final follow-up rate was very high at the conclusion of the third year. Second, we did not include the frequency of emergency department (ED) visits as one of the criteria of asthma exacerbation in this study. In Japan, pulmonary physicians often prescribe oral steroids for patients to be used in case of an exacerbation. Thus, we think that the frequency of ED visits may not necessarily indicate the actual frequency of exacerbations. Third, we did not measure airway hyperresponsiveness, which could be a significant factor associated with exacerbation. Lastly, we used the older definition of severe asthma that was developed during the ATS workshop in 2000¹⁴ since the new definition reported in the ERS/ATS guidelines¹⁵ had not yet been officially announced when this study was

designed. Nevertheless, as mentioned in the Materials and Methods section, we were certain that all subjects who were under appropriate treatment required high doses of ICS or OCS for asthma control.

In conclusion, through long-term follow-up and repeated measurements of a range of blood biomarkers, we were able to confirm that the frequent exacerbation phenotype of severe asthma appeared to be relatively stable over the 3-year study period. In addition, measurement of FeNO could be a valuable co-predictor for the management of severe asthma when combined with "past exacerbation status."

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CONFLICT OF INTERESTS

The authors HK, SK, HM, NT, KS, MS, HK, HG, YN, JO, SO, and YI declare no conflict of interest. MS has received grants from GlaxoSmithKline, AstraZeneca, and Novartis Japan outside the submitted work. KI has acted as a paid consultant to Chugai Pharmaceutical Co. Ltd and has received funding for research carried out in this work. KI also received research funding from Shino-test Co. Ltd and Chugai Pharmaceutical Co. Ltd. SEW has received grants from AstraZeneca, Boehringer Ingelheim, GlaxoSmithKline, Sanofi-Genzyme, and Novartis. SEW also received consulting fee or honorarium from AstraZeneca, Boehringer Ingelheim, Sanofi Genzyme, Novartis, and Merck. MN has received grants from Japan Allergy Foundation, AstraZeneca, and Kyorin Pharmaceutical Co. during the conduct of the study.

ORCID

S. Konno  <http://orcid.org/0000-0001-6439-2443>

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Figure E1

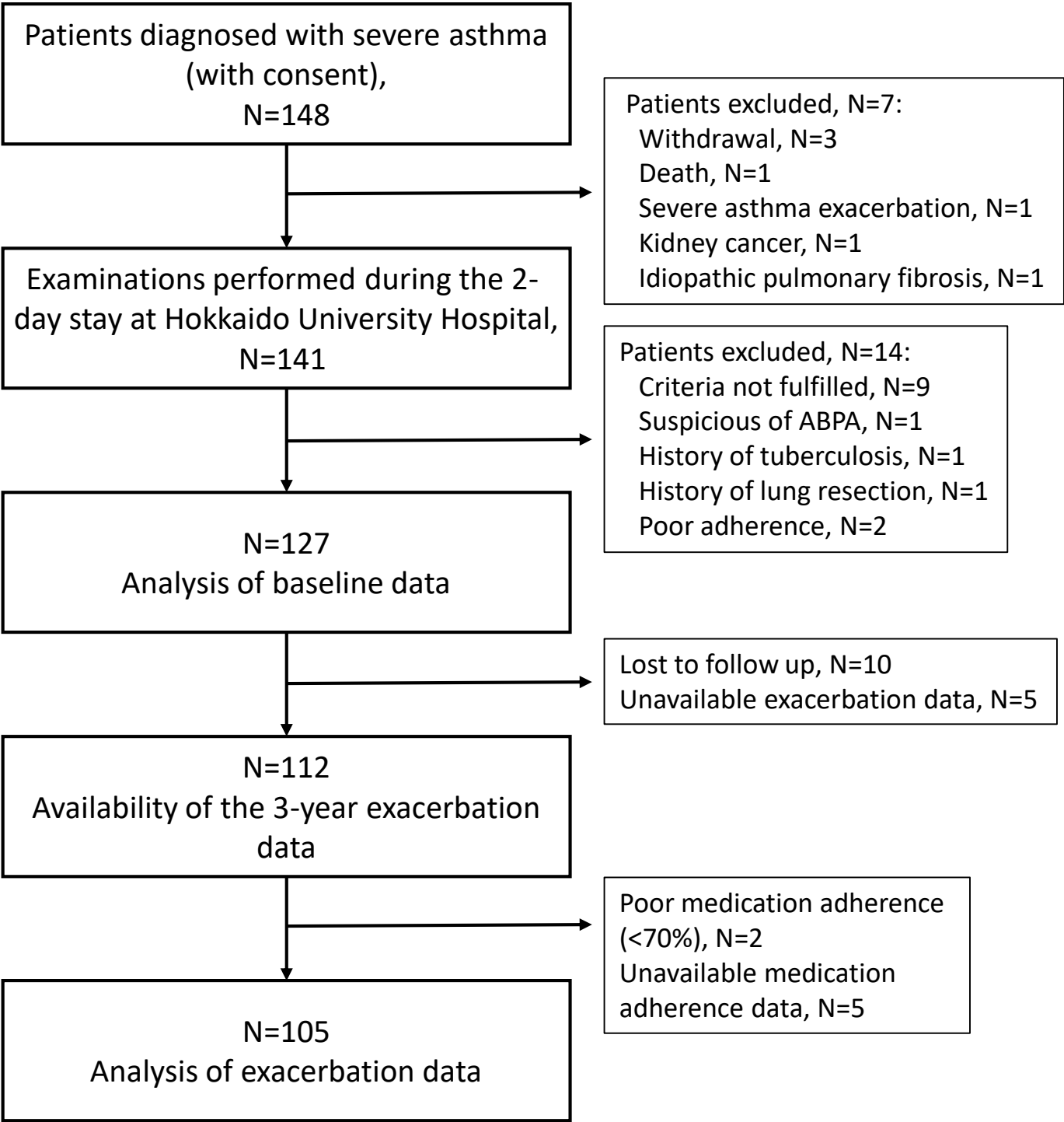


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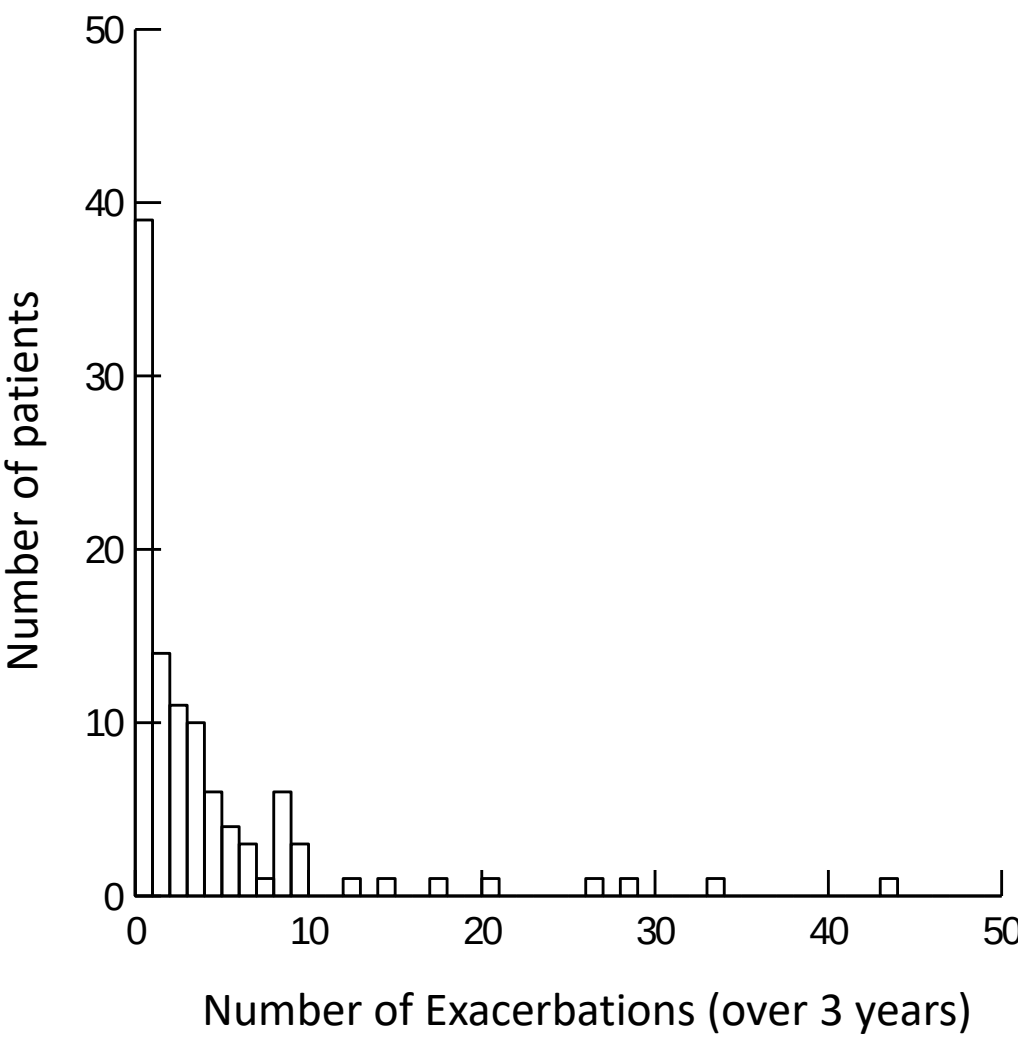
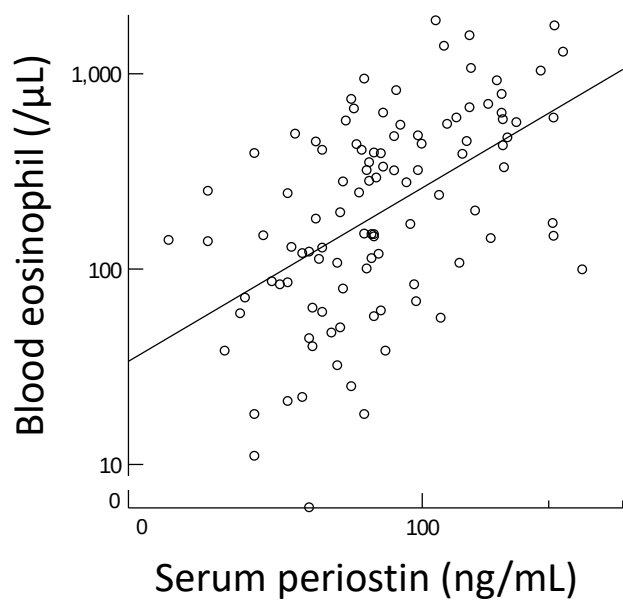
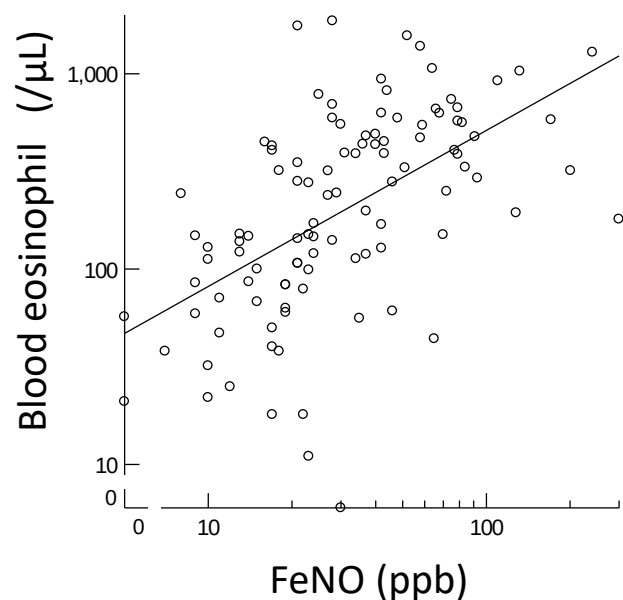


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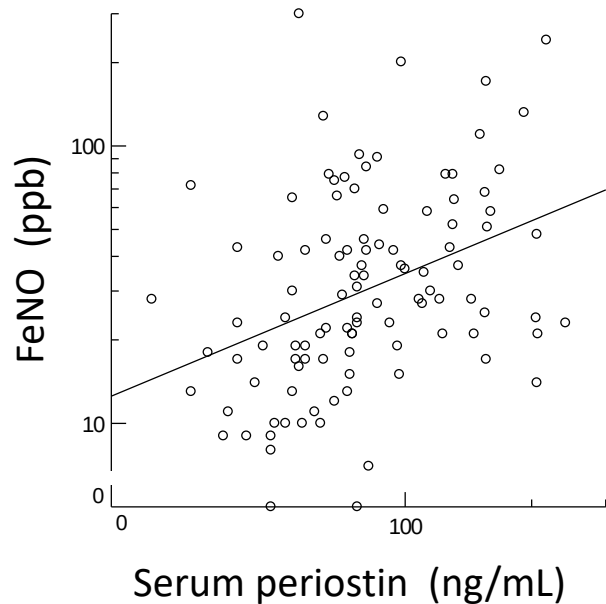
(A)



(B)



(C)



(D)

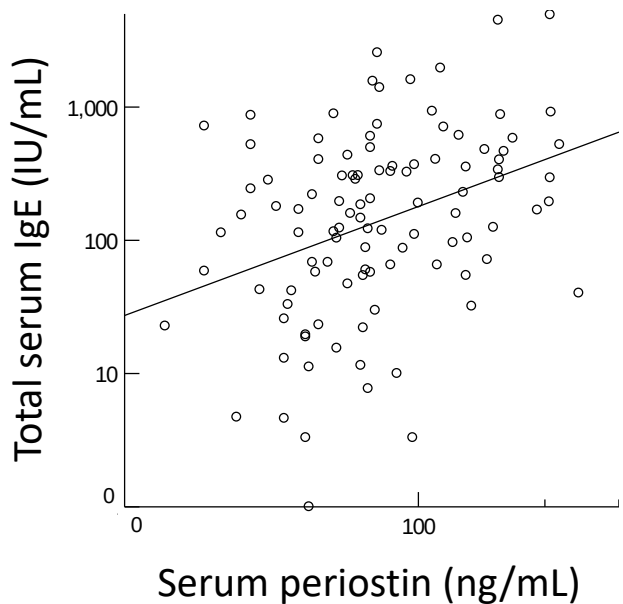


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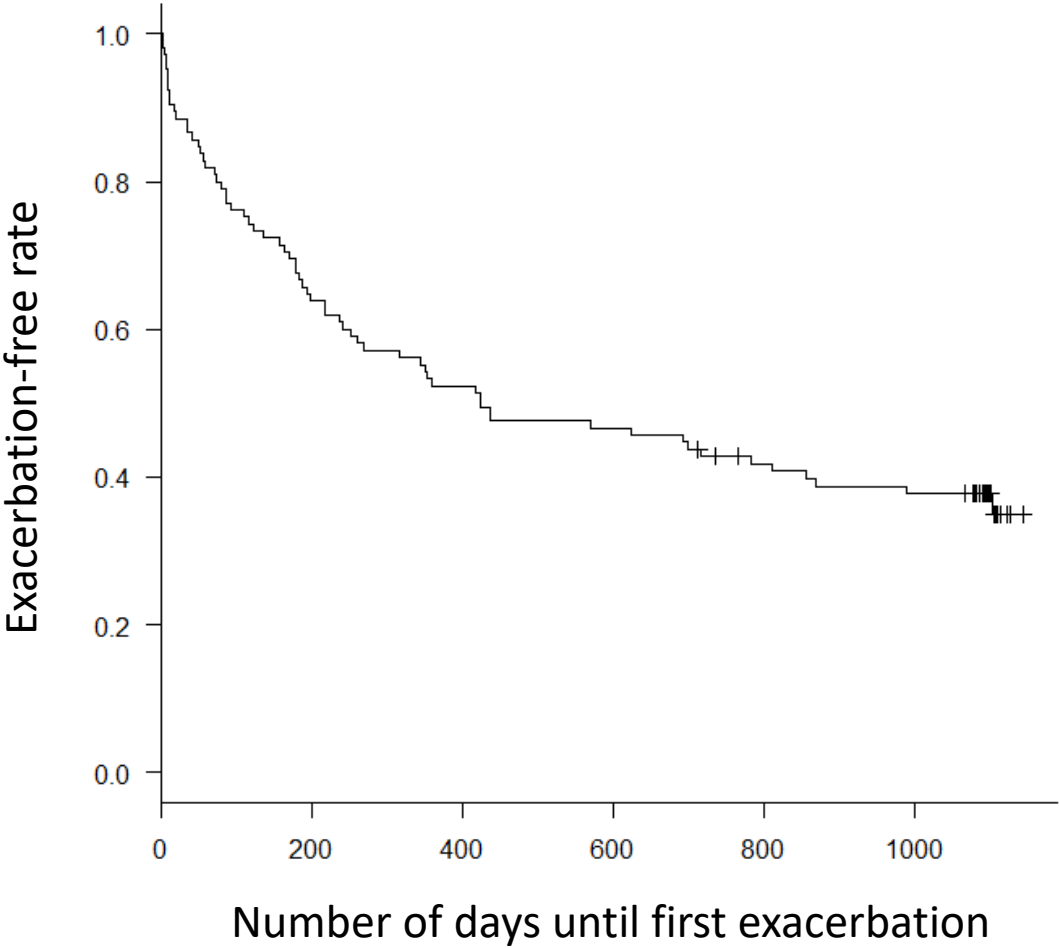


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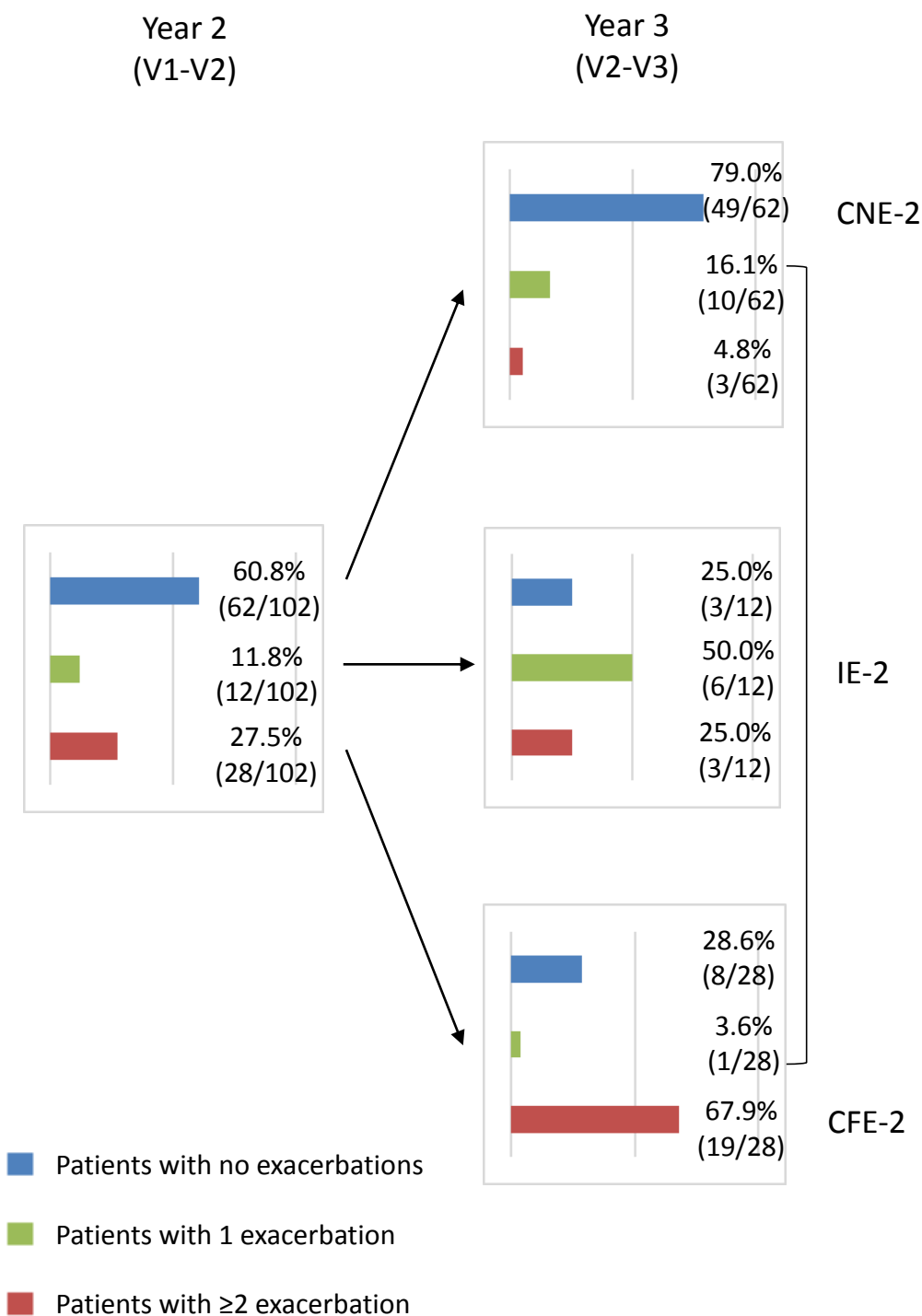
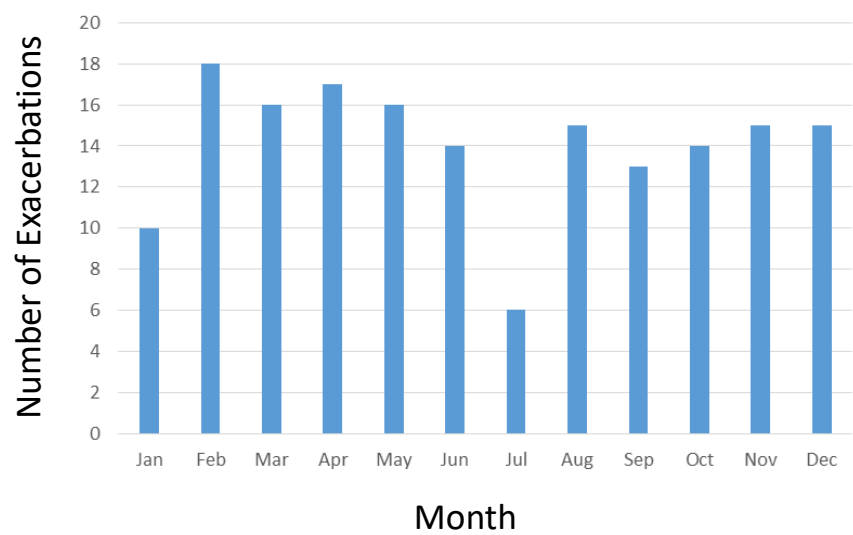
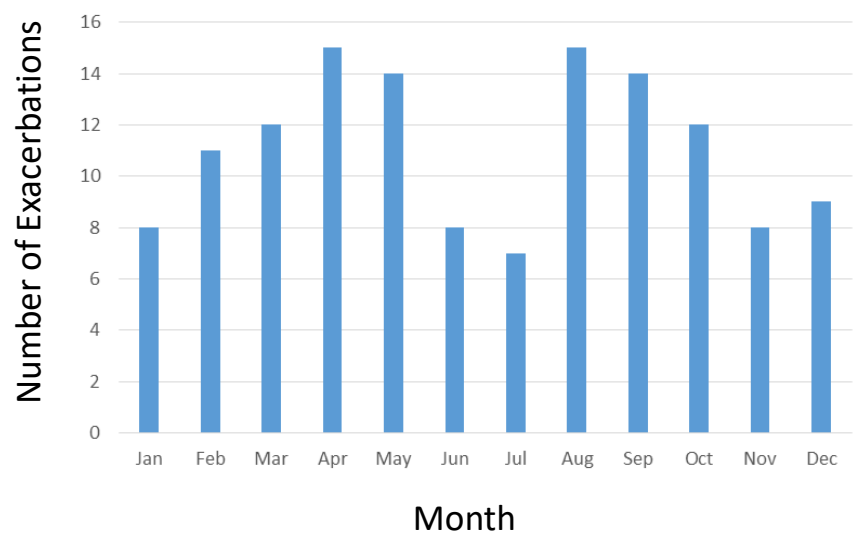


Figure E6

(A) Exacerbations in Year 1 (VE-V1)



(B) Exacerbations in Year 2 (V1-V2)



(C) Exacerbations in Year 3 (V2-V3)

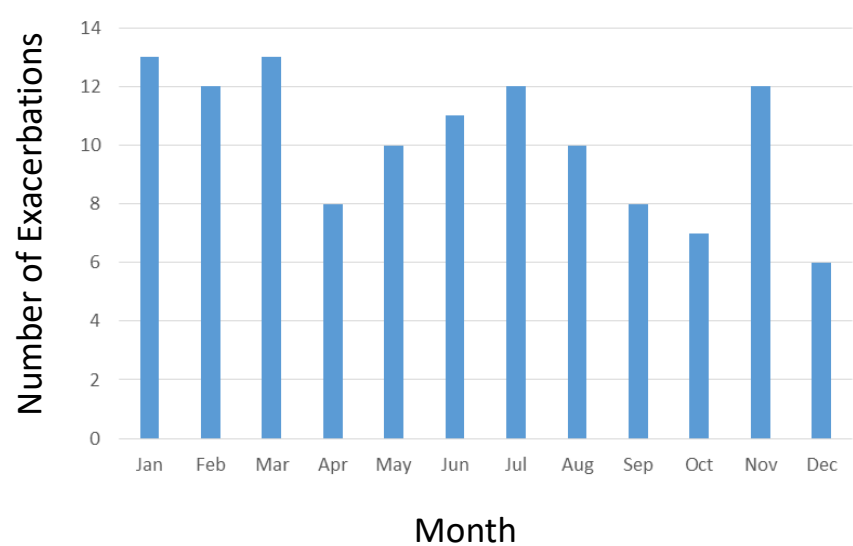
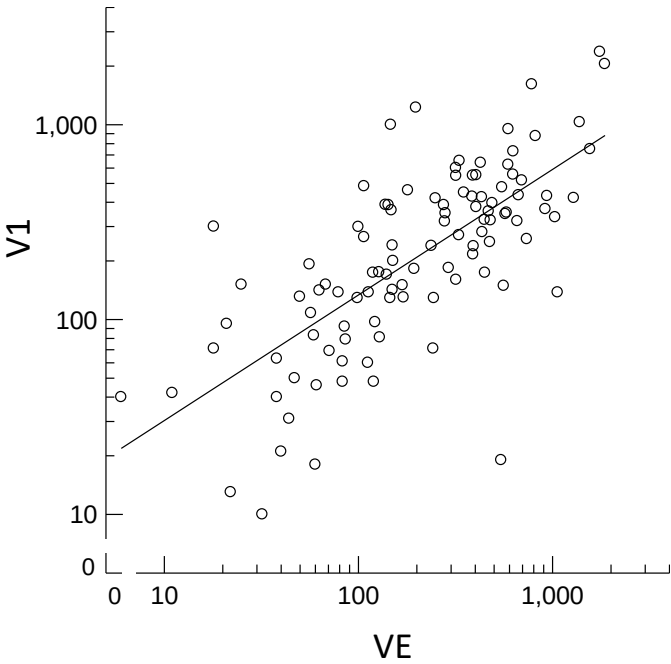
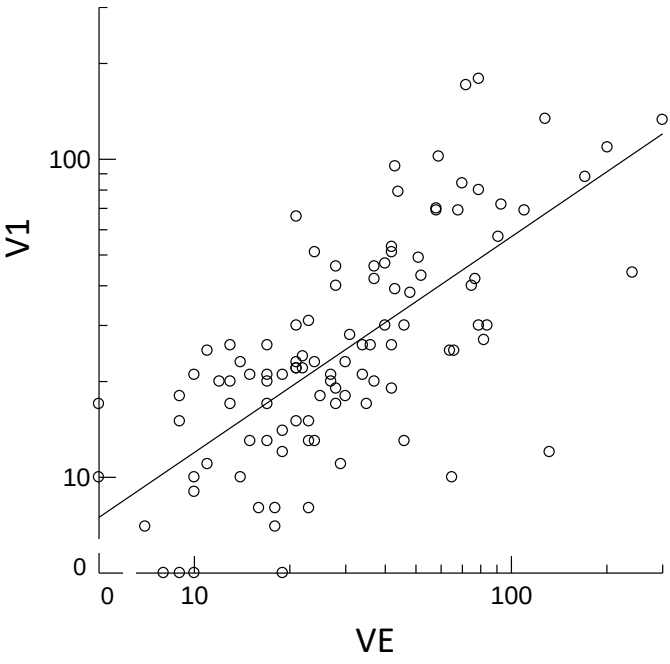


Figure E7

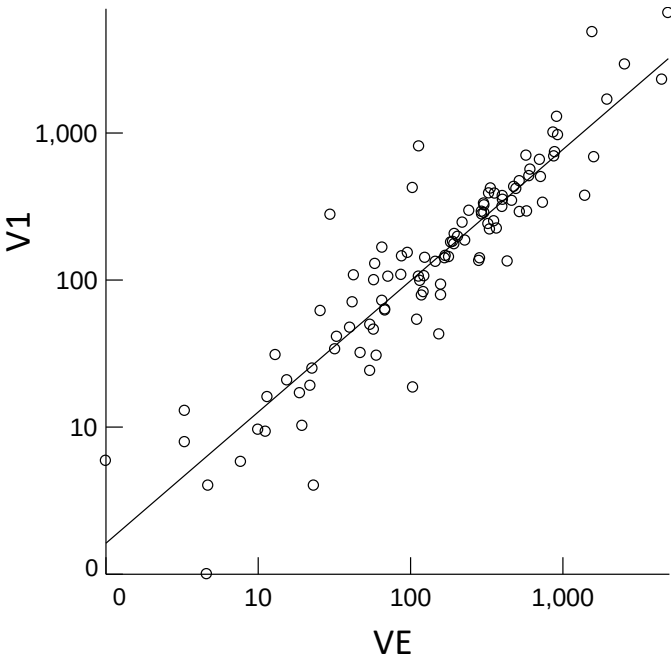
(A) Blood eosinophil (/ μ L)



(B) FeNO (ppb)



(C) Total serum IgE (IU/mL)



(D) Serum periostin (ng/mL)

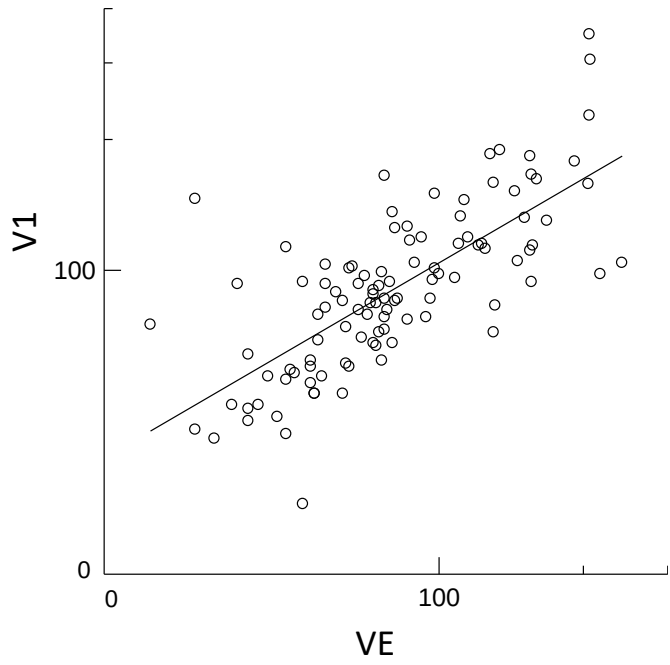
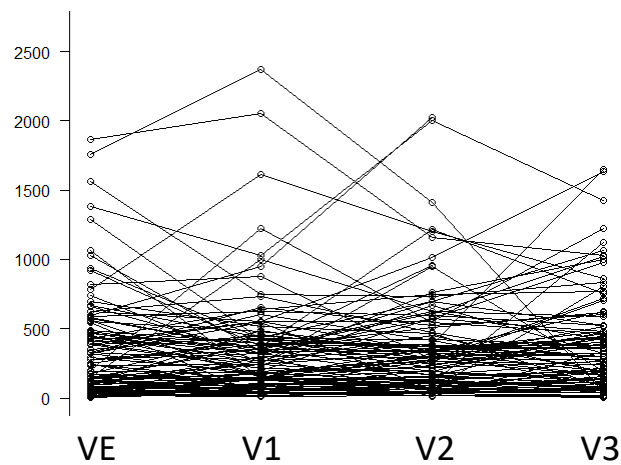
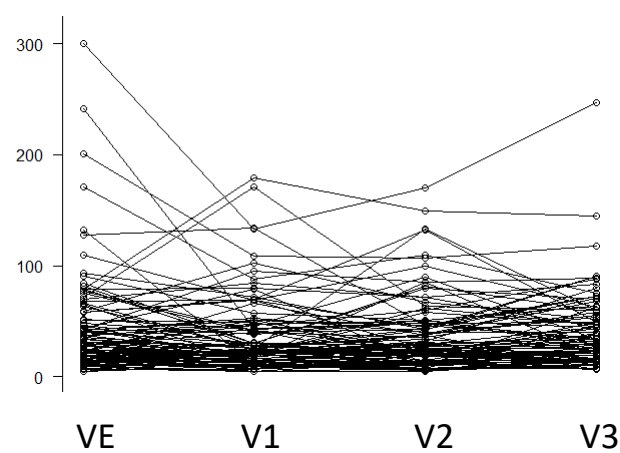


Figure E8

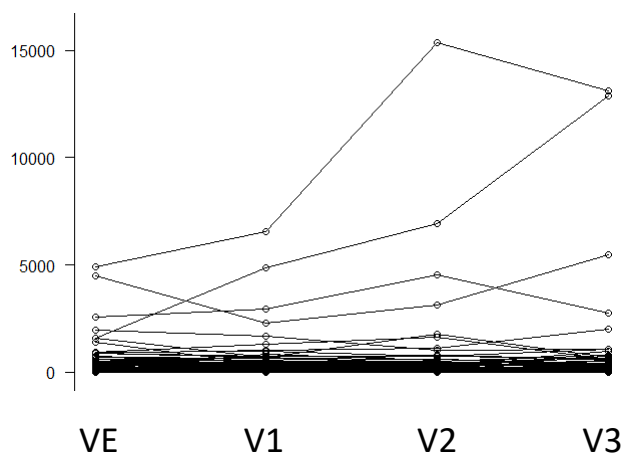
(A) Blood eosinophil (/μL)



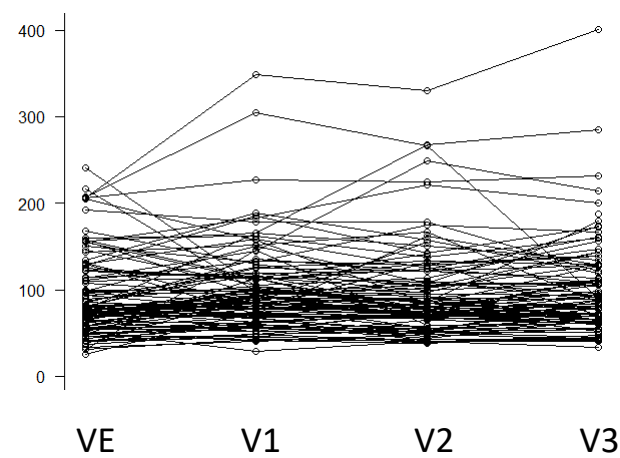
(B) FeNO (/ppb)



(C) Total serum IgE (IU/mL)



(D) Serum periostin (ng/mL)



Prospective predictors of exacerbation status in severe asthma over a 3-year follow-up

Hirokazu Kimura, MD, PhD 1, Satoshi Konno, MD, PhD 1, Hironi Makita, MD, PhD 1, Natsuko Taniguchi, MD, PhD 1, Kaoruko Shimizu, MD, PhD 1, Masaru Suzuki, MD, PhD 1, Hiroki Kimura, MD, PhD 1, Houman Goudarzi, MD, PhD 1, Yuji Nakamaru, MD, PhD 2, Junya Ono, BSc 3, Shoichiro Ohta, MD, PhD 4, Kenji Izuhara, MD, PhD 4, Yoichi M. Ito, PhD 5, Sally E. Wenzel, MD, PhD 6, Masaharu Nishimura, MD, PhD 1, and for the Hi-CARAT investigators

1 Department of Respiratory Medicine, Faculty of Medicine and Graduate School of Medicine, Hokkaido University

2 Otolaryngology Head and Neck Surgery, Hokkaido University School of Medicine

3 Shino-Test Corporation

4 Division of Medical Biochemistry, Department of Biomolecular Sciences, Saga Medical School

5 Department of Biostatistics, Faculty of Medicine and Graduate School of Medicine, Hokkaido University

6 University of Pittsburgh Asthma Institute at UPMC/University of Pittsburgh School of Medicine

Online Data Supplement

Material and Methods

This study was approved by the ethics committees of Hokkaido University Hospital and its 29 affiliated hospitals and clinics, and all subjects provided their written informed consent. This study was registered in the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) system (https://upload.umin.ac.jp/cgi-open-bin/ctr/ctr_view.cgi?recptno=R000003917; ID no. 000003254). The details of the Materials and Methods employed in this study are described in our previous reports (E1).

1. Participants

We enrolled patients who were diagnosed with severe asthma by respiratory physicians between February 2010 and September 2012 at the Hokkaido University Hospital and its 29 affiliated hospitals and clinics. A total of 141 subjects with severe asthma underwent procedural evaluations during a two-day stay at the Hokkaido University Hospital. After confirmation of the inclusion criteria, adherence to medications, and inhalation technique, a total of 127 patients with severe asthma were selected for baseline analyses (E1). The study inclusion and exclusion criteria were described in our previous report (E1). Several clinical parameters were evaluated in all subjects during their two-day stay at Hokkaido University Hospital (E1).

2. Definition of asthma and severe asthma

The diagnosis of asthma was made by respiratory physicians according to the Global Initiative for Asthma (GINA) criteria (E2) on the basis of recurrent respiratory symptoms with demonstrable reversible airflow limitation. At some time during the course, variable airflow limitation was confirmed based on excessive bronchodilator reversibility or/and significant increase in forced expiratory volume in one second (FEV₁) after treatment with controller medications (i.e., an increase in FEV₁ > 12% and > 200 mL). Severe asthma was diagnosed based on the American Thoracic Society (ATS) criteria of refractory asthma published in 2000 (E3), with slight modifications, as follows:

Major Characteristics

In order to achieve asthma control:

1. Treatment with continuous or near continuous (>50% of year) oral corticosteroids
2. Requirement for treatment with high-dose inhaled corticosteroids: the inhaled corticosteroid doses were modified due to differences in their availability across Japan, as follows: Flutide® ≥800 µg, Pulmicort® ≥1200 µg, QVAR® ≥600 µg, Alvesco® ≥600 µg, Asmanex® ≥600 µg, Adoair® ≥1000 µg, and Symbicort® ≥960 µg.

Minor Characteristics

1. Requirement for daily treatment with a controller medication in addition to inhaled corticosteroids, e.g., long-acting β-agonist, theophylline, or leukotriene antagonist

- 55 2. Asthma symptoms requiring short-acting β -agonist use on a daily or near daily basis
- 56 3. Persistent airway obstruction ($FEV_1 < 80\%$ predicted; diurnal PEF variability $> 20\%$)
- 57 4. One or more urgent care visits for asthma per year
- 58 5. Three or more oral steroid “bursts” per year
- 59 6. Prompt deterioration with $< 25\%$ reduction in oral or inhaled corticosteroid dose
- 60 7. Near fatal asthma event in the past

61 The diagnosis of severe asthma required one or both major criteria and two minor criteria. Patients who
62 were well-controlled under the current medications (absence of any of the minor characteristics 2, 4, or 5 upon
63 study entry) were asked if within one year they experienced episodic deterioration of symptoms, urgent care
64 visits, and rescue use of short-acting bronchodilators when the dose of the current medication was reduced.

65

66 **3. Clinical evaluations**

67 Upon entry to the study and admission to Hokkaido University Hospital, all subjects underwent evaluation for
68 several clinical parameters.

69

70 **3-1. Pulmonary function test**

71 Upon study entry and during each hospital visit, spirometry was performed before and after inhalation of 400
72 μ g oxitropium and 400 μ g salbutamol. The maximum forced expiratory volume in one second (FEV_1)
73 obtained from the two procedures and the FEV_1/FVC (forced vital capacity) value that corresponded to the

maximum FEV₁ value were used for the analyses. Owing to the severity of asthma in all subjects, respiratory medicines were not prohibited prior to the procedure, except for the use of short-acting bronchodilators for at least 12 hours prior to all measurements. Pulmonary function tests were performed, as described in our previous report (E1).

3-2. Questionnaires

Clinical research coordinators (CRCs) directly administered comprehensive questionnaires to all subjects on day 1 of admission. The questionnaires were administered to obtain the following information:

i. Age at the onset of asthma

The age of the patient at the onset of asthma was self-reported. To ensure accuracy, patients were asked to describe their experiences during episodes of dyspnea, wheezing, or coughing during childhood and puberty. In cases of uncertainty, the time of the earliest respiratory symptoms was designated as the age of onset of asthma symptoms and was used to calculate the duration of asthma.

ii. Smoking status

The smoking status of all patients was assessed by the CRCs. We classified subjects into two groups: smokers [≥ 10 pack-years (PY)] and nonsmokers (< 10 PY), according to the number of cigarette packs based on our previously reports (E1).

93 iii. Asthma quality of life questionnaire

94 In all participants, the asthma-related quality of life (QOL) was assessed using the Japanese version of the
95 Asthma Quality of Life Questionnaire (AQLQ) developed by Juniper (E4, E5). The AQLQ consisted of 32
96 items separated into four sections: symptoms, activity limitations, emotional function, and exposure to
97 environmental stimuli. The overall score of the AQLQ was calculated as the mean of all items. Permission to
98 use the Japanese version of the AQLQ was obtained from Dr. Juniper.

99

100 iv. Gastroesophageal reflux disease

101 The severity of gastroesophageal reflux disease (GERD) was assessed using the frequency scale for the
102 symptoms of GERD (FSSG) (E6). The FSSG consisted of 12 questions that were scored to indicate the
103 frequency of symptoms as follows: never = 0, occasionally = 1, sometimes = 2, often = 3, and always = 4.
104 Patients with scores ≥ 8 were considered to have GERD.

105

106 v. Depression

107 The severity of depression was assessed using the Self-Rating Depression Scale (SDS) (E7, E8). The SDS
108 consisted of 20 questions that were scored to indicate the frequency of symptoms as follows: never = 0,
109 occasionally = 1, sometimes = 2, often = 3, and always = 4. Patients with scores ≥ 50 were considered to have
110 depression.

111

vi. Rhinitis symptoms

The presence of rhinitis symptoms was assessed using questionnaires according to the Practical Guideline for the Management of Allergic Rhinitis in Japan 2009 (E9).

vii. Aspirin-exacerbated respiratory disease (AERD)

The presence of AERD was diagnosed by each chief respiratory physician and confirmed by the CRCs, based on the histories of intolerance to non-steroidal anti-inflammatory drugs (NSAIDs).

3-3. Measurement of biomarkers

The peripheral blood eosinophil count was obtained from the standard complete blood count, which was performed by the Laboratory Division of Hokkaido University Hospital. Total serum immunoglobulin E (IgE) levels (IU/mL) and specific IgE responses to common inhaled allergens, including *Dermatophagoides farinae*, grass pollens, animal dander, and molds, were determined. Atopy was defined based on positive multiple-antigen simultaneous test scores (lumicount > 1.01) to at least one common inhaled allergen (E1, E10). Blood and sputum supernatant samples were immediately frozen after collection and stored at -80°C until assay.

Serum periostin levels were measured with an enzyme-linked immunosorbent assay (ELISA) at Shino-test laboratory (Kanagawa, Japan), as described previously (E1, E11). Sputum was induced by inhalation of 4.5% saline via an ultrasonic nebulizer, as described previously (E1).

131

132 **4. Protocol of the three-year follow-up**

133 **4-1. Assessment of exacerbation**

134 We gave all participants an “asthma diary” to collect exacerbation information. When systemic corticosteroids
135 for an asthma attack were administered in the hospitals/clinics, we asked the doctors to record in the patient’s
136 asthma diary the dose and the total number of days in which systemic steroids were used. When oral
137 corticosteroids (OCS) for an asthma attack were used by the patients themselves at home, the patients were
138 instructed to record this information their diaries. In this case, the chief doctor carefully checked the diary
139 during each patient’s visit to the hospital/clinic to determine if their asthma was worsening. During their
140 yearly visits to the Hokkaido University Hospital, the patients were directly questioned by the clinical
141 research coordinator (CRC) and a doctor regarding the number of exacerbation episodes within one year, and
142 the CRC/doctor confirmed the patients’ responses in the diary records.

143

144 **4-2. Assessment of adherence to medications**

145 During the follow-up period, we asked all subjects to record their medication use in their asthma diary for four
146 weeks every season (a total of 16 weeks per year). We also asked the patients to send their diaries to the
147 Hokkaido Respiratory Cohort Office for verification by the CRCs. The patients visited each hospital/clinic
148 every 1-2 months and the chief doctor confirmed adherence to their medications at every visit. For the patients
149 who visited Hokkaido University once a year, the CRCs and doctors (Dr. HK, SK, KS, YM, HK) directly

inquired regarding the patients' adherence.

5. Statistical analysis

To compare the differences among the groups, the Student's t-test or one-way analysis of variance (ANOVA) was used to evaluate the continuous parametric variables, the Mann-Whitney U test was used for the continuous non-parametric variables, and the chi-square test was used for the categorical variables. In the multivariate analyses, we considered age, sex, body mass index (BMI), atopic status, smoking status [pack year (PY) > 10] (E1), use of oral corticosteroids (OCS), and the presence of AERD (Model 1, Figure 1). In addition, generalized estimating equations (GEEs) were applied to calculate odds ratios for the associations of several variables with repeated measures of exacerbation status (Model 2, Figure 1). To evaluate the correlation between two parameters, the Pearson correlation was used. Factors associated with exacerbation-free survival were analyzed using the Kaplan–Meier method with the Cox proportional hazards model. To analyze the tendency of the continuous variables and categorical among the three exacerbation groups, we performed the Jonckheere–Terpstra trend test or the Cochran-Armitage trend test.

Results

Association of asthma exacerbation and seasonality

As shown in Figure E6, the season in which exacerbations occurred more frequently seems visually different in each year. During the first year, the number of exacerbations was lower in July, and during the second year, it was lower in June and July. However, the trend shown during the first and second years was not observed during the third year. Thus, seasonality was not consistently associated with exacerbation tendency.

Factors associated with the number of days until the first asthma exacerbation

Figure E4 shows the Kaplan–Meier curve demonstrating the number of days until the first asthma exacerbation over the three-year study period. Results from the Cox proportional hazards model revealed that only FeNO concentration (obtained at VE) was significantly associated with the first exacerbation [Analysis 1, Crude; $P = 0.034$, Model 1; $P = 0.018$] (Table E4). When this analysis was focused on periods after one year (from V1 to V3, Analysis 2), FeNO obtained at V1 showed a significant association (Crude; $P = 0.006$, Model 1; $P = 0.004$, Model 2; $P = 0.004$) (Table E10).

Stability of the Th2-related biomarkers over the three-year follow-up period

As shown in Figure E7, there were strong correlations between the data from the initial visit and the one-year follow-up visit for all of the following parameters: peripheral blood eosinophil level ($R = 0.708$, $P < 0.0005$),

183 total serum IgE level ($R = 0.915$, $P < 0.0005$), FeNO concentration ($R = 0.698$, $P < 0.0005$), and serum
184 periostin level ($R = 0.674$, $P < 0.0005$). Figure E8 depicts line graphs of the Th2-related biomarkers over the
185 three-year study period (VE, V1, V2, and V3). The blood eosinophil level, total serum IgE level, FeNO
186 concentration, and serum periostin level were relatively stable at each of the 4 assessments.

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- E1. Kimura H, Konno S, Nakamaru Y, Makita H, Taniguchi N, Shimizu K, et al. Sinus Computed Tomographic Findings in Adult Smokers and Nonsmokers with Asthma. Analysis of Clinical Indices and Biomarkers. *Ann Am Thorac Soc* 2017;14:332-41.
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Table E1. Medication adherence data of the study subjects

Adherence (%)	Year 1 (VE-V1)			Year 2 (V1-V2)			Year 3 (V2-V3)		
	Oral Med.	Inhaled Med.	Transdermal Med.	Oral Med.	Inhaled Med.	Transdermal Med.	Oral Med.	Inhaled Med.	Transdermal Med.
99-100	80 (72.1%)	75 (67.0%)	4 (50.0%)	82 (78.1%)	69 (63.3%)	6 (66.7%)	78 (76.5%)	74 (69.2%)	4 (57.1%)
90-99	27 (24.3%)	30 (26.8%)	3 (37.5%)	20 (19.0%)	38 (34.9%)	2 (22.2%)	20 (19.6%)	31 (29.0%)	2 (28.6%)
80-90	2 (1.8%)	6 (5.4%)	0	2 (1.9%)	1 (0.9%)	1 (11.1%)	3 (2.9%)	2 (1.9%)	1 (14.3%)
70-80	0	1 (0.9%)	1 (12.5%)	1 (1.0%)	1 (0.9%)	0	1 (1.0%)	0	0
0-70	2 (1.8%)	0	0	0	0	0	0	0	0
All	111	112	8	105	109	9	102	107	7

Abbreviations: Med, medication; VE, Visit Entry; V1, Visit 1-year; V2, Visit 2-year; V3, Visit 3-year.

Table E2. Factors associated with frequent exacerbation during Year 2 (from V1 to V2)

	OR	95% CI	P-value
Male sex	1.31	0.40-4.31	0.661
Age*	0.98	0.93-1.03	0.421
Duration*	1.02	0.99-1.06	0.248
Atopic status	1.28	0.39-4.19	0.681
AERD	1.32	0.33-5.28	0.691
BMI*	0.99	0.89-1.11	0.886
PY $\geq$ 10*	1.10	0.34-3.56	0.876
Frequent exacerbations in Year 1 (from VE to V1)	8.85	3.14-25.0	<0.0001

*at V1

Abbreviations: AERD, aspirin-exacerbated respiratory disease ; BMI, body mass index; CI, confidence interval; OR, odds ratio; PY, Pack years; VE, Visit Entry; V1, Visit 1-year; V2, Visit 2-year.

Logistic regression analysis.

Table E3. Factors associated with frequent exacerbation during Year 3 (from V2 to V3)

	OR	95% CI	P-value
Male sex	1.22	0.29-5.08	0.782
Age*	0.98	0.92-1.04	0.494
Duration*	1.00	0.96-1.04	0.889
Atopic status	1.62	0.39-6.75	0.505
AERD	1.75	0.38-8.12	0.472
BMI*	1.00	0.88-1.13	0.960
PY $\geq$ 10*	2.19	0.55-8.69	0.266
Frequent exacerbation in Year 1 (from VE to V1) and Year 2 (from V1 to V2)	35.3	8.09-153.8	<0.0001

*at V2

Abbreviations: AERD, aspirin-exacerbated respiratory disease ; BMI, body mass index; CI, confidence interval; OR, odds ratio; PY, Pack years; VE, Visit Entry; V1, Visit 1-year; V2, Visit 2-year; V3, Visit 3-year.

Logistic regression analysis.

Table E4. The association of blood eosinophil and FeNO with exacerbation-free survival (Cox proportional hazards model) (Analysis 1)

	HR	95% CI	P-value
Blood eosinophil *			
Crude	1.51	0.91-2.49	0.110
Adjusted †	1.56	0.93-2.61	0.090
FeNO *			
Crude	1.99	1.06-3.76	0.034
Adjusted †	2.42	1.16-5.04	0.018

Abbreviations: CI, confidence interval; FeNO, fractionated exhaled nitric oxide; HR, hazards ratio.

* Log-transformed.

† Model 1 (see Figure 1) Adjusted for age, asthma duration, sex, body mass index, atopic status, smoking status (PY $\geq$ 10), oral corticosteroid use, and aspirin-exacerbated respiratory disease.

Table E5. The subjects' characteristics at V1 according to their exacerbation status after V1 (Analysis 2)

	All (N = 102)	Type of exacerbation			P-value
		CNE-2 (N = 49)	IE-2 (N = 34)	CFE-2 (N = 19)	
Male sex, N (%)	42 (41.2%)	20 (40.8%)	14 (41.2%)	8 (42.1%)	0.995
Age at V1, years	59.5 ± 12.2	59.1 ± 11.3	60.6 ± 13.6	58.3 ± 12.6	0.770
Asthma duration, years	20.6 ± 14.6	18.3 ± 12.3	23.1 ± 16.7	22.0 ± 16.1	0.310
Smoking status (Current/Ex/Never)	12/52/38	6/23/20	6/17/11	0/12/7	0.361
Pack years	5.5 (0-22.0)	5.3 (0-22.3)	7.0 (0-24.0)	4.0 (0-11.4)	0.606
Pack years ≥10, N (%)	43 (42.2%)	19 (38.8%)	15 (44.1%)	9 (47.4%)	0.781
Body mass index, kg/m ²	25.5 ± 4.8	25.3 ± 5.3	26.5 ± 3.3	24.2 ± 5.4	0.253
Daily ICS dose, µg *	1598.0 ± 586.1	1596.9 ± 552.9	1654.4 ± 592.2	1500.0 ± 673.1	0.659
Maintenance OCS use, N (%)	36 (35.3%)	15 (30.6%)	12 (35.3%)	9 (47.4%)	0.431
Atopy, N (%)	63 (61.8%)	33 (67.3%)	18 (52.9%)	12 (63.2%)	0.410

Abbreviations; CFE-2, consistent frequent exacerbators in analysis 2; CNE-2, consistent non-exacerbators in analysis 2; FeNO, fractional exhaled nitric oxide; ICS inhaled corticosteroid; IE, intermittent exacerbators in analysis 2; OCS, oral corticosteroids; V1, Visit 1-year.

Data are shown as the mean ± standard deviation (SD), median (interquartile range), geometric mean (log₁₀ SD), or number (%).

P-values were obtained using a chi-square test, one-way analysis of variance (ANOVA), or the Mann-Whitney U-test, as appropriate.

* Equivalent to budesonide dose.

Table E6. Characteristics of the biomarkers, pulmonary function tests, and AERD at Visit 1, according to exacerbation status after Visit 1 (Analysis 2)

	All (N = 102)	Type of exacerbation			P-value	P for trend*
		CNE-2 (N = 49)	IE-2 (N = 34)	CFE-2 (N = 19)		
Biomarkers						
Blood eosinophil, cells/μL	202.7 (0.47)	190.6 (0.50)	181.7 (0.46)	289.2 (0.38)	0.281	0.463
Blood neutrophil, cells/μL	4674.4 (0.18)	4173.0 (0.18)	5379.1 (0.16)	4871.6 (0.19)	0.021	0.061
Total serum IgE, IU/mL	129.8 (0.68)	145.9 (0.68)	102.1 (0.79)	147.7 (0.43)	0.549	0.539
FeNO, ppb	24.2 (0.33)	19.8 (0.27)	26.1 (0.36)	35.3 (0.36)	0.014	0.0027
Serum periostin, ng/mL	89.8 (0.19)	91.4 (0.23)	80.6 (0.11)	104.3 (0.17)	0.105	0.595
Pulmonary function tests						
FEV ₁ , % predicted (maximum) †	87.9 ± 17.9	87.4 ± 18.3	88.0 ± 18.3	89.0 ± 17.3	0.947	0.843
FEV ₁ /FVC, % ‡	65.5 ± 13.1	65.4 ± 13.3	64.7 ± 12.9	67.2 ± 13.5	0.806	0.823
AERD	14 (13.7%)	4 (8.2%)	4 (11.8%)	6 (31.6%)	0.039	0.021
COPD §	61 (59.8%)	29 (59.2%)	20 (58.8%)	12 (63.2%)	0.946	0.815

Abbreviations: AERD, aspirin-exacerbated respiratory disease; CFE-2, consistent frequent exacerbators in analysis 2; CNE-2, consistent non-exacerbators in analysis 2; FeNO, fractional exhaled nitric oxide; FEV₁, forced expiratory volume in one second; FVC, forced vital capacity; ICS inhaled corticosteroid; IE-2, intermittent exacerbators in analysis 2; OCS, oral corticosteroids.

Data are shown as the mean $\pm$ standard deviation (SD), geometric mean ($\log_{10}$ SD), or number (%).

P-values were obtained using a chi-square test or one-way analysis of variance (ANOVA), as appropriate.

* Jonckheere-Terpstra trend test or Cochran-Armitage trend test.

† Maximum value of FEV₁ among the four procedures (see Methods).

‡ FEV₁/FVC was applied using the value corresponding to the maximum FEV₁.

§ Defined as FEV₁/FVC < 70%.

Table E7. Comparison of the blood eosinophil count and FeNO among exacerbation status groups in two-year follow-up after Visit 1 (Analysis 2)

	Type of exacerbation			P-value Crude	P-value Model 1	P-value Model 2
	CNE-2 (N = 49)	IE-2 (N = 34)	CFE-2 (N = 19)			
Blood eosinophil, cells/ μ L *	190.6 (0.50)	181.7 (0.46)	289.2 (0.38)	0.281	0.300	0.522
FeNO, ppb *	19.8 (0.27)	26.1 (0.36)	35.3 (0.36)	0.014	0.007	0.009

Abbreviations: AERD, aspirin-exacerbated respiratory disease; CFE-2, consistent frequent exacerbators in analysis 2; CNE-2, consistent non-exacerbators in analysis 2; FeNO, fractional exhaled nitric oxide; IE-2, intermittent exacerbators in analysis 2.

Data are shown as the geometric mean ($\log_{10}$ standard deviation).

P-values were obtained using a one-way analysis of variance (ANOVA).

* Log-transformed.

Model 1: Adjusted for age, asthma duration, sex, body mass index, atopic status, smoking status ($\text{PY} \geq 10$), oral corticosteroid use, and AERD.

Model 2: Adjusted for factors in Model 1 and “exacerbation status during the first year”.

Table E8. Factors associated with consistently frequent exacerbations during Year 2 and 3 (Analysis 2)

	OR	95% CI	P-value
Male sex	1.00	0.24-4.23	>0.999
Age*	1.00	0.94-1.06	0.953
Duration*	1.01	0.97-1.06	0.573
Atopic status	1.46	0.33-6.40	0.613
AERD	3.75	0.75-18.8	0.109
BMI*	0.91	0.78-1.06	0.242
PY $\geq$ 10*	1.69	0.41-6.96	0.466
FeNO*	10.3	1.27-83.9	0.029
Frequent exacerbations in Year 1 (from VE to V1)	13.1	3.32-51.3	0.0002

*at V1

Abbreviations: AERD, aspirin-exacerbated respiratory disease; BMI, body mass index; CI, confidence interval; FeNO, fractionated exhaled nitric oxide; OR, odds ratio; PY, Pack years; VE, Visit Entry; V1, Visit 1-year.

Logistic regression analysis.

Table E9. Factors associated with consistently frequent exacerbations during Year 2 and 3
(Analysis 2: using generalized estimating equations)

	OR	95% CI	P-value
Male sex	1.08	0.36-3.20	0.893
Age*	0.98	0.95-1.02	0.434
Duration*	1.02	0.99-1.06	0.153
Atopic status	1.43	0.52-3.91	0.485
AERD	2.07	0.57-7.43	0.267
BMI*	0.98	0.88-1.09	0.715
PY $\geq$ 10*	1.50	0.53-4.20	0.442
FeNO*	8.62	2.06-36.0	0.003
Frequent exacerbations in Year 1 (from VE to V1)	11.7	4.70-29.3	<0.0001

*at V1

Abbreviations: AERD, aspirin-exacerbated respiratory disease; BMI, body mass index; CI, confidence interval; FeNO, fractionated exhaled nitric oxide; OR, odds ratio; PY, Pack years; VE, Visit Entry; V1, Visit 1-year.

ORs, CIs and P-values are calculated by using generalized estimating equations.

Table E10. The association of blood eosinophil and FeNO with exacerbation-free survival after V1 (Cox proportional hazards model) (Analysis 2)

	HR	95% CI	P-value
Blood eosinophil *			
Crude	1.31	0.71-2.39	0.389
Adjusted (Model 1) †	1.43	0.74-2.77	0.287
Adjusted (Model 2) ‡	1.18	0.61-2.29	0.630
FeNO *			
Crude	3.53	1.45-8.63	0.006
Adjusted (Model 1) †	4.02	1.58-10.2	0.004
Adjusted (Model 2) ‡	4.20	1.56-11.3	0.004

Abbreviations: CI, confidence interval; FeNO, fractionated exhaled nitric oxide; HR, hazards ratio; V1, Visit 1-year.

* Log-transformed.

† Model 1 (see Figure 1) Adjusted for age, asthma duration, sex, body mass index, atopic status, smoking status (PY $\geq$ 10), oral corticosteroid use, and aspirin-exacerbated respiratory disease.

‡ Model 2 (see Figure 1) Adjusted for factors in Model 1 and exacerbation status during the first year.

On-line Figure legends

Figure E1.

Flow chart depicting the subject selection for this 3-year study.

Abbreviations: ABPA, Allergic bronchopulmonary aspergillosis

Figure E2.

Number of exacerbations that occurred during the three-year follow-up period.

Figure E3

Correlations between the blood/sputum biomarkers.

(A) Blood eosinophil and serum periostin levels.

(B) Blood eosinophil and FeNO concentration.

(C) FeNO concentration and serum periostin level.

(D) Total serum IgE)and serum periostin levels.

Figure E4

Kaplan–Meier curves for exacerbation-free survival over the three-year follow-up period.

Figure E5

Stability of the exacerbation frequency status after one-year (from V1 to V3) (Analysis 2)

Figure E6

The number of exacerbation events occurring in each month during the follow-up period.

(A) During first year (from VE to V1)

(B) During second year (from V1 to V2)

(C) During third year (from V2 to V3)

Figure E7

Correlations between the data obtained VE and V1.

(A) Blood eosinophil

(B) FeNO concentration

(C) Total serum IgE level

(D) Serum periostin level

Figure E8

Line graphs of the biomarkers during the three-year follow-up period.