Total IgE Variability Is Associated with Future Asthma Exacerbations: A 1-Year Prospective Cohort Study

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BACKGROUND: Few prospective studies have investigated the relationship between IgE variability and risk for asthma exacerbations (AEs).
OBJECTIVE: To explore the relationship between IgE variability and AEs.
METHODS: Recruited patients with stable asthma underwent two serum total IgE tests within a month (at screening [baseline IgE] and at 1 month) to obtain the coefficient of variation (CV) of base 10 log-transformed IgE. Patients with IgE CV were divided into IgE CV-high and IgE CV-low cohorts based on the CV median and were observed within 12 months, during which the association between IgE variability and AEs was explored using a negative binomial regression model.

RESULTS: The IgE CV levels obtained from 340 patients classified patients into two groups (n [170 for the IgE CV-high and IgE CV-low groups, respectively) based on the serum total IgE CV median of 2.12% (quartiles 1 and 3: 0.98% and 3.91%, respectively). The IgE CV-high patients exhibited worse asthma control and lung function and more marked airway inflammation, and received more intensive medication use compared with IgE CV-low patients. The IgE CV-high patients exhibited increased rates of moderate-to-severe (adjusted rate ratio [2.88; 95% confidence interval, 1.65-5.03; P< .001) and severe (adjusted rate ratio [2.16; 95% confidence interval, 1.08-4.32;
P[.029) AEs during the follow-up year compared with IgE CV-low patients. Furthermore, sputum IL-6 partially mediated the associations between IgE CV with moderate-to-severe and severe AEs.
CONCLUSIONS: Variability in total serum IgE levels is an easily obtained and practical measure for predicting AEs. Studies are needed to investigate whether IgE variability can be used to guide precision medicine in asthma. (J Allergy Clin Immunol Pract 2021;9:2812-24)
Key words: Asthma; IgE; Variability; Exacerbation; Cohort study

INTRODUCTION

Asthma exacerbations (AEs) can have serious consequences that require hospitalization and may even be fatal, and are associated with significant health care costs.1,2 Asthma exacerbation occurs in the general population of asthmatic patients and is not confined to those with more severe disease.3 In 2019, approximately 15% of people with asthma required hospitalization in China.4 There are 500,000 hospitalizations per year owing to AEs, costing nearly $5 billion annually in the United States.5 To prevent exacerbations and their detrimental consequences, it is important to identify patients who are at risk.
IgE is a characteristic feature of type I hypersensitivity and a key trigger for allergic airway inflammation in asthma.6 Previous studies reported its critical roles in the pathophysiology of allergic and nonallergic asthma.7-9 Serum total IgE levels have exhibited correlations with asthma control, lung function, and asthma severity.10-12 However, evidence of the utility of serum total IgE levels as a predictive biomarker for AE is limited. Semprini et al13 observed that elevated baseline IgE levels were negatively associated with future AEs in severe refractory asthma; however, this was not observed in patients with mild and moderate asthma. Similarly, the Severe Asthma Research Program (SARP)-3 cohort identified an inverse relationship between baseline IgE levels and frequent AEs, which could not be replicated in either the SARP1 or SARP-2 cohorts.14 These irreproducible results indicate that a single measured IgE value is an insufficient predictor of future AEs.
Several studies discovered evidence of intrasubject variability in total IgE levels as a function of time, which may be related to AEs.15,16 In a retrospective cohort study, Tanaka et al17 observed that patients with increased levels of serum total IgE experienced more AEs than did patients with decreased or unchanged IgE levels. However, IgE variability in that study was defined arbitrarily by total IgE at the time of the study minus total IgE detected 10 years earlier. There is no reference standard method for assessing the variability of IgE levels. Previous studies recommended that the definition of peak expiratory flow variability in patients with asthma should be based on the coefficient of variation (CV).18,19 The CV represents the ratio of the SD to the mean19 and is a useful statistic for comparing the

degree of variation from one data series to another; thus, it may be a reliable means of determining IgE variability.

In this study, CV was used to determine the serum total IgE variability, which was associated with asthma-related clinical and inflammatory characteristics. Then, we explored whether total IgE variability was associated with future AEs in the following year. Finally, post-hoc mediation analyses were used to explore the underlying inflammatory mechanism of the relationship between IgE CV and AEs.

The certain findings of this study were previously presented as an abstract.20

METHODS Study design and patients

This was a real-world, 1-year prospective cohort study designed to explore the relationship of IgE variability with AEs. This study was composed of two parts. In the enrollment phase, patients were observed for 1 month to determine the base 10 log-transformed (lg) serum total IgE CV. The median of serum total lg (IgE) CV in patients was used as the cutoff value to divide patients into two cohorts: lg (IgE) CV-high or lg (IgE) CV-low cohort (1-month cohort). Then, these patients were observed up for 12 months (face-to-face visits or telephone calls, if unavailable) to assess the occurrence of AEs (Figure 1). Asthma exacerbationerelated details, including date of occurrence, treatment, and health care provider, were recorded in an investigator-led questionnaire. The case report form in this study was based on the Australasian Severe Asthma Network,21 some data of which were previously published.22-24 Ethical approval for the study was obtained from the institutional review board at West China Hospital of Sichuan University (Chengdu, China) (No. 2014-30), and written consent was obtained from all patients. This cohort study was registered as ChiCTROOC-16009529 (http://www.chictr.org.cn).

Patients aged greater than 18 years, with stable asthma, were consecutively recruited from the Asthma Clinic of West China Hospital at Sichuan University (Chengdu, China) between January 2015 and May 2019. Asthma was determined by respiratory physicians based on the diagnostic features of a history of both variable respiratory symptoms and variable airflow obstruction.25 Variable airflow obstruction was confirmed by evidence of either airway hyperresponsiveness or bronchodilator responsiveness with more than a 12% and 200-mL increase from baseline in forced expiratory volume in 1 second (FEV1).25 Stable asthma was defined as an exacerbation-free condition with no respiratory infection, or no change in maintenance therapy in the preceding 4 weeks.26 Patients who were pregnant or breastfeeding, or who had other chronic unstable diseases were excluded.

As a real-world study, indications for patient treatment were based on the Global Initiative for Asthma recommendations.25 Stepup or step-down treatments were adjusted in a continuous cycle of assessment, treatment, and review.

Clinical measurements and data collection

Comprehensive clinical data were collected, including demographic and clinical characteristics such as age, sex, medications, family history of asthma, and atopic status.22-24 Asthma control and quality of life were assessed using the Asthma Control Test (ACT) score and Asthma Quality of Life Questionnaire, respectively, which have been validated in the Chinese population.27,28 Asthma control was determined by the ACT score, in which scores of 20-25 were classified as well-controlled asthma, 16-19 as partially controlled asthma, and 5-15 as uncontrolled asthma. Atopic status was determined by at least one positive skin prick test for common local seasonal and perennial allergens, as previously described.25,29 In our study, seasonal allergens30 were composed of mixed tree pollen (birch and London plane) and mixed grass pollen (ragweed, humulus, and Artemisia annua),

whereas perennial allergens31 included house dust mites, dog and cat hair, and Alternaria. The fraction of exhaled nitric oxide32 (FENO) (NIOX analyzer, Aerocrine, Solna, Sweden) and spirometry33 (MedGraphics Corp, St. Paul, MN) were measured based on the American Thoracic Society/ European Respiratory Society recommendations. In addition, all subjects underwent blood sampling and sputum induction.

Blood processing

Fasting venous blood samples, in either ethylenediamine tetraacetic acid-treated or untreated tubes, were collected. Blood samples were used to test total and differential white blood cell counts (Sysmex XN-9000 hematologic analyzer, Sysmex Corporation, Kobe, Japan), as well as serum total IgE (immune scatter turbidity, Beckman Image 800 immunoassay analyzer, Beckman Coulter Inc, Brea, Calif). All patients underwent two serum total IgE tests within a month: at screening (baseline IgE) and 1 month (29-31 days)34 later. The minimum detectable level of total IgE was 5.0 IU/mL.

Sputum processing and inflammatory cytokine testing

Sputum induction and processing were performed based on a protocol described in our previous published study.35 Briefly, sputum was induced using either 4.5% hypertonic saline or saline atomized with an ultrasonic nebulizer (Cumulus, HEYER Medical AG, Rhineland-Palatinate, Germany) in subjects with FEV1% predicted 40% or greater or FEV1% predicted less than 40%. Sputum plugs (100 mL) were processed using 400 mL dithiothreitol and 400 mL phosphate-buffered saline. Cytospins were prepared using a centrifugation-smear (CytoPro 7620, Wescor, Inc, Logan, UT), whereas stained (May-Grunwald-Giemsa) and differential cell counts were obtained from 400 nonsquamous cells. Differential cell counts were performed by well-trained researchers from the both the University of Newcastle, New South Wales, Australia, and West China Hospital, China.

The sputum supernatant was aspirated and frozen at 80C to test IFN-gamma (lower limit of detection [LLD], 0.8 pg/mL), IL-1b (LLD, 0.8 pg/mL), IL-4 (LLD, 4.5 pg/mL), IL-5 (LLD, 0.5 pg/mL), IL-6 (LLD, 0.9 pg/mL), IL-8 (LLD, 0.8 pg/mL), IL-13 (LLD, 1.3 pg/mL), and TNF-a (LLD, 0.8 pg/mL) by Luminex-based MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel Kit (EMD Millipore Corporation, Burlington, Mass). Spiking experiments of cytokines in sputum supernatants showed that recovery ranged from 70% to 130% for all detectable analyses.36

Asthma phenotypes

Patients were classified into four inflammatory phenotypes37: eosinophilic asthma (induced sputum eosinophils 3% or greater alone and/or blood eosinophils 0.3 109 cells/L or greater), neutrophilic asthma (neutrophils 61% or greater and eosinophils less than 3% in induced sputum), mixed granulocytic asthma (eosinophils greater than 3% and neutrophils greater than 61% in induced sputum), and paucigranulocytic asthma (neutrophils less than 61% and eosinophils less than 3% in induced sputum). Allergic asthma was defined as positive reaction to common allergens using skin prick test.25,29 Late-onset asthma was defined as asthma onset at age 12 years or greater.38 Type 2-high asthma was defined as meeting two of three criteria: a total IgE level of more than 100 IU/ mL, eosinophil count 0.14 109 cells/L or greater, and FENO of 30 parts per billion or greater.39,40

Definition of total IgE variability

Serum total IgE values were base 10 log-transformed to obtain a normal distribution. The CV was calculated to define serum total lg IgE variability: the ratio of the standard deviation to the mean of two measurements of lg IgE19:

Primary and secondary outcomes

Primary and secondary outcomes were moderate-to-severe AEs and severe AEs, respectively, during follow-up. Asthma exacerbation was defined based on the American Thoracic Society/European Respiratory Society statement.41 Therefore, moderate AE was defined as a deterioration in symptoms and lung function and increased rescue bronchodilator use, with a duration of 2 or more days, but not severe enough to warrant systemic corticosteroid use.41 Severe AE was defined as systemic corticosteroid use lasting at least 3 days for acute asthma, or an asthma-related hospitalization or emergency department visit owing to AE requiring systemic corticosteroids for at least 3 days.41 For consistency, courses of corticosteroids separated by 1 week or more were treated as separate severe exacerbations.41

Statistical analysis

Categorical variables are presented as frequencies and proportions, and continuous variables are expressed as means (SD) or median (interquartile range). When possible, all continuous data were transformed into a normal distribution. The difference between cohorts for each variable was evaluated using Student t test or MannWhitney U test for continuous variables and chi-square test or Fisher exact test for categorical variables, as appropriate.

We used logistic regression analysis to assess associations between single sputum cytokines and IgE CV with or without adjusting for age, sex, body mass index (BMI), smoking status, FEV1% predicted, inhaled corticosteroid (ICS) dosage, and baseline IgE concentrations. Negative binomial regression models were used to explore the association of IgE variability with AEs during the follow-up year, adjusting for confounders including sex, age, BMI, smoking status, atopic status, FEV1% predicted, ICS dosage, baseline IgE concentrations, and AEs in the previous year.14,18 Subgroup analysis was performed to explore the relationship between IgE variability and AEs in subpopulations. We further tested the predictive value of baseline IgE and mean IgE levels to AEs using negative binomial regression models owing to the potential clinical significance of baseline IgE and mean IgE levels at baseline and 1 month later for AE prediction (see Methods in this article's Online Repository at www.jaci-inpractice.org).

FIGURE 1. Flowchart of study. AE, asthma exacerbation; CV, coefficient variation; lg, logarithm base 10.

We used post-hoc parallel multiple mediation models to examine whether the relationship of IgE CV (a dichotomous independent variable, IgE CV-high vs IgE CV-low, X) with AEs (dependent variable, Y) was mediated by sputum cytokines (mediator variables, M) (Figure 2, A). Mediation hypotheses were tested using biascorrected bootstrapping with 10,000 samples to calculate 95% confidence intervals (CIs) with the PROCESS macro (version 3.3) for SPSS (version 22.0, SPSS, Chicago, IL) using ordinary leastsquares regression to estimate variables on the left side of the model equations.42 Significance was achieved when an indirect effect (a b) was observed and zero was not included in the CIs, instead of individual tests of the paths of the model (c, a, and b).42 Detailed information is provided in the Methods in the Online
Repository.

Statistical analysis was performed using IBM SPSS software (version 22.0). All tests were two-tailed; $P < .05$ was considered statistically significant.

RESULTS

Demographic and clinical characteristics A total of 347 patients were included in baseline testing. During the 1-month follow-up, seven patients were excluded because they experienced an AE (n ¼ 3), were unable to make contact (n ¼ 1), or refused to participate (n ¼ 3) without l g (IgE) CV. Finally, IgE CV levels were successfully obtained from 340 patients who were classified into two groups (n ¼ 170 for the IgE CV-high and IgE CV-low groups, respectively) based on the median of serum total IgE CV of 2.12% (quartiles [Q]1 and Q3, 0.98% and 3.91%). Then, these two cohorts were prospectively observed for 12 months, in which 27 patients were excluded owing to an inability to make contact (n ¼ 10) or a refusal to participate (n ¼ 17). Therefore, 313 patients (IgE CV-high cohort, n ¼ 157; IgE CV-low cohort, n ¼ 156) were included in the final analysis (Figure 1).
Compared with patients in IgE CV-low cohort, those in the IgE CV-high cohort had worse asthma control (IgE CV-high vs CV-low: ACT scores, 19.00 [15.00-22.00] vs 20.00 [16.75-22.25]; P ¼ .012)(2.07, worse lung function presented as 0.78 vs 2.21 0.763.27L; lower capacity of FEV1
P ¼ .047) and forced vital capacity (3.03 [2.42-3.60] vs
510.65 vs 539.36medication (beclomethasone dipropionate equivalent: 608.28[2.71-3.73] L; P ¼ 482.93.035), and received more intensive ICSmg/d; P ¼ .019) (Table I).
In terms of asthma phenotypes, the IgE CV-high cohort had a lower proportion of patients with T2-high (IgE CVhigh vs CV-low: 35.3% vs 64.7%; P < .001) and allergic (48.2% vs 67.6%; P < .001) asthma but a higher proportion of late-onset asthma (87.6% vs 79.4%;IgE CV-low cohort. However, there were no differences be-P ¼ .041), than the tween cohorts in asthma inflammatory phenotype (P ¼ .775) (Table I).

FIGURE 2. Multiple parallel mediation model of association of IgE coefficient of variation (CV) with asthma exacerbations (AEs) using sputum cytokines as mediators after controlling for sex, age, body mass index, smoking status, atopic status, forced expiratory volume in 1 s % predicted, inhaled corticosteroid dosage and baseline total IgE level. (A) Conceptual parallel mediation model. Mediation model of association between (B) IgE CV-high and moderate-to-severe AEs or (C) severe AEs with sputum IL-6 and IL-8 as mediator. a ¼ effect of X on M; b ¼ effect of M on Y; c0 ¼ direct effect of X on Y controlling for M; ab ¼ indirect effect of X on Y through M (the product of path coefficients a and b); c ¼ total effect of X on Y (direct and indirect). Each path is presented as unstandardized coefficients (b) with standard errors. *P < .05. BC, bias-corrected; CI, confidence interval; IE, indirect effect.
Compared with patients in the IgE CV-low cohort, those in the IgE CV-high cohort had lower levels of total IgE both at baseline (IgE CV-high vs CV-low: 88.34 [32.56-221.03] vs 207.75 [82.57-463.39] IU/mL; P < .001) and 1 month later (80.61 [32.71-171.75] vs 202.87 [79.91-444.78] IU/mL; P < .001). Moreover, patients in the IgE CV-high cohort had lower proportions of sensitization of seasonal or perennial allergens than did patients in the IgE CV-low cohort (both P < .05)
(Table I).
Local and systemic inflammation
Patients in the IgE CV-high cohort had significant airway inflammation characterized by higher levels of sputum IL-6 and IL-8 compared with patients in the IgE CV-low cohort (IgE CV-high vs CV-low: 20.84 [8.92-51.88] vs 13.66 [5.20-32.56] pg/mL; P ¼ .019; and 1337.00 [808.83-3106.00] vs 1108.00 [579.67-2218.00] pg/mL; P ¼ .028; respectively) (Figure 3). However, there were no significant differences in either counts or percentages of sputum

granulocytes (including total cells, neutrophils, eosinophils, macrophages, and lymphocytes) and FENO (all P > .05) between the IgE CV-high and IgE CV-low cohorts
(Table II).
Furthermore, we compared inflammatory cells in the peripheral blood between cohorts. Patients in the IgE CV-high cohort had more neutrophils (IgE CV-high vs CV-low: 3.86 [2.87-4.92] 109 cells/L vs 3.38 [2.82-4.15] 109 cells/L; P ¼ .027) and a lower percentage of eosinophils (3.50% [1.73% to 6.28%] vs 4.40% [2.34% to 6.87%]; P ¼ .036) in the blood than did patients in IgE CV-low cohort
(Table II).
TABLE I. Demographic and clinical characteristics of patients grouped by total logarithm base 10 (IgE) coefficient of variation (CV) within
1 mo*

Characteristic	IgE CV-high	IgE CV-low	t/Z/c2	P
n	170	170		
0.713				
.476{				
Age, y (median [Q1-Q3])	44.33 (34.89-55.55)	43.57 (34.93-53.79)		
Female, n (%)	108 (63.5)	109 (64.1)	0.013	.910#
Body mass index, kg/m2 (median [Q1-Q3])	23.10 (20.70-25.10)	23.22 (21.22-25.25)		
0.539	.590{			
Smoking, pack-y (mean [SD])	3.1 (8.9)	2.6 (8.6)	1.811	
.070{				
Atopic status, n (%)	82 (48.2)	115 (67.6)	13.143	.001#
Seasonal allergensz, n (%)	21 (12.4)	36 (21.2)	4.742	
.029#				
Perennial allergensx, n (%)	61 (35.9)	79 (46.5)	3.934	.047#
Seasonal and perennial allergens, n (%)	9 (5.3)	22 (12.9)		
5.999	.014#			
Family history, n (%)	61 (35.9)	66 (38.8)	0.044	.835#
Age of onset, y (median [Q1-Q3])	34.00 (22.00-46.00)	32.00 (20.00-44.00)	1.330	.184#
Asthma Control Test score (median [Q1-Q3])	19.00 (15.00-22.00)	20.00 (16.75-22.25)	2.525	.012{
Asthma Quality of Life Questionnaire score (median [Q1-Q3])	5.72 (5.00-6.35)	5.90 (5.35-6.37)	1.732	
.083{				
HADS-A (median [Q1-Q3])	2 (0-4)	1 (0-3)	1.548	.122{
HADS-D (median [Q1-Q3])	1 (0-3)	1 (0-4)	1.707	.088{
Sinusitis, n (%)	79 (46.5)			
608.28 (510.65)	85 (50.0)			
539.36 (482.93)	0.384			
2.341	.535#			
.019{				

Medications
Inhaled corticosteroid dosage, beclomethasone equivalent, mg/d (mean [SD])

Inhaled corticosteroid with long-acting b-agonist, n (%) 95 (55.9) 100 (58.8) 0.301 .583#
Leukotriene receptor antagonist, n (%) 154 (90.6) 150 (88.2) 0.497 .481#
Oral corticosteroid, n (%) 7 (4.1) 5 (2.9) 0.346 .557#
Global Initiative for Asthma steps 4 and 5, n (%) 36 (21.2)

2.06 (0.78) 44 (25.9)

2.21 (0.76) 1.046

1.988 .306#

.047{
Spirometry
FEV1, L (mean [SD])
FEV1, % predicted (median [Q1-Q3]) 72.00 (56.00-88.00) 73.00 (29.50-89.00) 0.780 .435{
Forced vital capacity, L (median [Q1-Q3]) 3.03 (2.42-3.60) 3.27 (2.71-3.73) 2.107 .035{
FEV1 forced vital capacity, % (mean [SD]) 65.14 (13.74) 66.55 (14.05) 0.553 .349k
Asthma phenotypes
Inflammatory phenotypes†, eosinophilic asthma/ neutrophilic asthma/paucigranulocytic asthma, n (%) 58 (42.6)/25 (18.4)/53 (39.0) 56 (44.4)/18 (14.3)/52 (41.3) 0.510 .775#
Late-onset/early-onset asthma, n (%) 149 (87.6)/21 (22.4) 135 (79.4)/35 (10.6) 4.190 .041#
Type 2-high/non-type 2 high asthma, n (%) 60 (35.3)/110 (64.7) 102 (60.0)/68 (40.0) 21.392 <.001#
Allergic/nonallergic asthma, n (%) 76 (44.7)/94 (55.3) 115 (67.6)/55 (32.4) 13.362 <.001#
Logarithm base 10 (IgE) CV, % (median [Q1-Q3]) 3.90 (2.83-6.19) 0.98 (0.39-1.43) 15.927 <.001{
Serum total IgE at screening, IU/mL (median [Q1-Q3]) 88.34 (32.56-221.03) 207.75 (82.57, 463.39) 4.968 <.001{
Serum total IgE 1 mo later, IU/mL (median [Q1-Q3]) 80.61 (32.71, 171.75) 202.87 (79.9-444.78) 5.578 <.001{

CV, coefficient of variation; FEV1, forced expiratory volume in 1 s; HADS-A, hospital anxiety and depression scale-anxiety; HADS-D, hospital anxiety and depression scaledepression; Q, quartile.
*Bold values denote statistical significance at P < .05.
†Mixed granulocytic asthma was not analyzed because only one patient was identified as having granulocytic asthma; eosinophilic asthma was defined as sputum eosinophils 3% or

greater alone and/or blood eosinophils 0.3 109 cells/L or greater; neutrophilic asthma was defined as sputum neutrophil count 61% or greater and eosinophil count less than 3%; paucigranulocytic asthma was defined as sputum neutrophil count less than 61% and eosinophil count less than 3%. zSeasonal allergens were composed of mixed tree pollen and mixed grass pollen. xPerennial allergens were composed of house dust mites, dog and cat hair, and Alternaria.
kStudent t test.
{Mann-Whitney U test.
#Chi-square test.

Associations of IgE variability with airway inflammatory profiles

We explored associations between sputum inflammatory cytokinesandIgECVusingalogisticregressionmodelbynotadjusting (Table E1 in this article's Online Repository at www.jaciinpractice.org) and adjusting (Table III) confounders including age, sex, BMI,smoking status, FEV1% predictive, ICSdosage, and baseline IgE concentrations. The levels of IL-6 (adjusted odds ratio

FIGURE 3. Comparison of airway inflammatory cytokines, including IFN (A), TNF-a (B), IL-1b (C), IL-4 (D), IL-5 (E), IL-6 (F), IL-8 (G), and IL13 (H) between the IgE coefficient of variation (CV)-high and IgE CV-low cohorts by Mann-Whitney U test.

[ORadj] ¼ 2.01; 95% CI, 1.10-3.66; P¼ .022) and IL-8 (ORadj ¼ 2.41; 95% CI, 1.08e5.39; P ¼ .032) in induced sputum were significantly associated with increased IgE variability. Although suggestive of an association, the result of the level of IL-1b and IgE CV did not achieve statistical significance (ORadj ¼ 1.62; 95% CI, 0.96-2.74; P ¼ .074).

TABLE II. Local and systemic inflammation in patients grouped by total logarithm base 10 (IgE) variability*

Variable	IgE coefficient of variation-high	IgE coefficient of variation-low	t/Z	P
n	170	170		
0.642 .127z				
.521z				
Fractional exhaled nitric oxide, parts per billion (median [Q1-Q3])	28.50 (16.75-66.0)			
9.37 (2.41-31.60)	40.00 (22.75-71.00)			
8.02 (2.86-27.45)				
Sputum				
Total cell count, 104/mL (median [Q1-Q3])				
Neutrophils				
104/mL (median [Q1-Q3])		0.098 .922z		
% (median [Q1-Q3])	37.38 (15.38-59.06)	28.25 (12.05-53.88)		
1.360 .174†				
Macrophages				
104/mL (median [Q1-Q3])	14.06 (3.59-26.54)	15.40 (6.01-30.33)		
0.709 .478z				
% (median [Q1-Q3])	52.13 (18.81-76.06)			

0.06 (0.00-0.67) 62.25 (28.94-82.56)

0.04 (0.00-0.46) 1.796 .073z
Eosinophils
104/mL (median [Q1-Q3]) 0.119 .905z
% (median [Q1-Q3]) 0.25 (0.00-3.00) 0.25 (0.00-1.19)
0.789 .430z
Lymphocytes
104/mL (median [Q1-Q3]) 0.23 (0.00-0.54) 0.25 (0.04-0.48)
0.468 .640z
% (median [Q1-Q3]) 0.50 (0.25-1.25)
0.50 (0.25-1.75)
0.363 .716z
Blood
Neutrophils
109/L (median [Q1-Q3]) 3.86 (2.87-4.92) 3.38 (2.82-4.15)
2.210 .027z
% (median [Q1-Q3]) 61.1 (54.1-66.1)

1.72 (1.45-2.17) 59.1 (53.2-63.6)

1.72 (1.36-2.05) 1.871 .061z
Lymphocytes
109/L (median [Q1-Q3]) 0.996 .319z
% (mean [SD]) 28.65 (7.23) 28.9 (6.71) 0.367 .714†
Monocytes
109/L (median [Q1-Q3]) 0.36 (0.27-0.49) 0.33 (0.28-0.43)
1.551 .121z
% (median [Q1-Q3]) 5.81 (4.88-7.04)

0.20 (0.10-0.40) 5.72 (4.87-6.96)

0.25 (0.14-0.42) 0.605 .545z
Eosinophils
109/L (median [Q1-Q3]) 1.312 .190z
% (median [Q1-Q3]) 3.50 (1.73-6.28) 4.40 (2.34-6.87)
2.095 .036z
Basophils
109/L (median [Q1-Q3]) 0.04 (0.02-0.06) 0.04 (0.02-0.05)
0.565 .572z
% (median [Q1-Q3]) 0.54 (0.37-0.82) 0.59 (0.41-0.83) 0.571
.568z

Q, quartile.
*Bold values denote statistical significance at P < .05.
†Student t test. zMann-Whitney U test.

Associations of IgE variability with AEs by negative binomial regression analyses
Moderate-to-severe AEs. Fifty patients experienced moderate-to-severe AEs (n ¼ 170; 16.0%) in the 1-year follow-up. Compared withpatientsinthe IgECV-low cohort,moremoderateto-severe AEs occurred in patients in the IgE CV-high cohort than in patients in the IgE CV-low cohort (n ¼ 17, 10.9% vs n ¼ 33, 21.0%; P ¼ .015). Also, frequencies of moderate-to-severe AE were greater in patients in the IgE CV-high cohort than in patients in the IgE CV-low cohort (0.48 1.26 vs 0.14 0.45, respectively; P ¼ .006) (Table IV).
Furthermore, the negative binomial regression analysis showed that IgE CV variability was significantly associated with moderateto-severe AEs (Figure 4). The rate of moderate-to-severe AEs was significantly higher for patients in the IgE CV-high cohort compared with those in the IgE CV-low cohort (adjusted rate ratio [RRadj] ¼ 2.88; 95% CI, 1.65-5.03; P < .001) after adjusting for age, sex, BMI, smoking status, atopic status, FEV1% predicted, ICS dosage, and baseline IgE level (Figure 4, A).
Subgroup analyses indicated that patients in the IgE-CV high cohort with T2-low (RRadj ¼ 4.13; 95% CI, 1.95-8.78; P < .001), late-onset asthma (RRadj ¼ 2.26; 95% CI, 1.78-5.97; P < .001), nonsmoking asthma (RRadj ¼ 2.45; 95% CI, 1.284.72; P ¼ .007), or well-controlled asthma (RRadj ¼ 3.63; 95% CI, 1.23-10.81; P ¼ .020) experienced significantly more moderate-to-severe AEs than did those in the IgE CV-low cohort. Surprisingly, the atopic status did not affect the association of IgE variability with AEs because patients with both allergic (RRadj ¼ 2.93; 95% CI, 1.35-6.35; P ¼ .006) and nonallergic (RRadj ¼ 2.65; 95% CI, 1.02-6.92; P ¼ .046) asthma in the IgE CV-high cohort had an increased risk for experiencing moderate-to-severe AEs when the IgE CV-low cohort was used as the reference (Figure 4, A).
Severe AEs. Thirty-seven patients experienced severe AEs in the 1-year follow-up (n ¼ 64; 11.8%) (Table IV). The IgE

TABLE III. Logistic regressions exploring associations of sputum cytokine with total logarithm base 10 (IgE) coefficient of variationhigh cohort*

Variable†	Adjusted odds ratio (95% confidence interval)z	P
IFN-gamma, pg/mL	0.16 (0.01-5.19)	.303
TNF-a, pg/mL	1.37 (0.73-2.59)	.330
IL-4, pg/mL	0.86 (0.58-1.28)	.465
IL-5, pg/mL	1.27 (0.56-2.88)	.572
IL-1b, pg/mL	1.62 (0.96-2.74)	.074
IL-6, pg/mL	2.01 (1.10-3.66)	.022
IL-8, pg/mL	2.41 (1.08-5.39)	.032
IL-13, pg/mL	1.35 (0.46-3.98)	.590

*Bold values denote statistical significance at P < .05. †Sputum cytokine values have been base 10 log-transformed.
zLogistic regressions exploring associations of sputum cytokine (independent vari-fi able) and total logarithm base 10 (IgE) coef cient of variation-high cohort (dependent variable) after adjusting for confounders, including sex, age, body mass index, smoking status, forced expiratory volume in 1 s % predictive, inhaled corticosteroid dosage, and baseline total IgE level.

TABLE IV. Asthma exacerbations in follow-up year grouped by logarithm base 10 (IgE) coefficient of variation (CV)*

Outcome	IgE CV-high	IgE CV-low	Z/c2/F	P
n	157	156		

Moderate-to-severe asthma exacerbation
n (%) 33 (21.0) 17 (10.9) 5.972 .015z
Mean (SD) 0.48 (1.26)

24 (15.3) 0.14 (0.45)

13 (8.3) 2.723 3.629 .006†

.057z
Severe asthma exacerbation
n (%)
Mean (SD) 0.31 (1.07) 0.10 (0.34) 1.987 .047†
Emergency department visits
n (%) 6 (3.8) 2 (1.3) .283x
Mean (SD) 0.11 (0.68)

11 (7.0) 0.01 (0.11)

7 (4.5) 1.692 0.916 .091†

.338z
Hospitalization
n (%)
Mean (SD) 0.10 (0.39) 0.05 (0.25) 0.972 .331†

*Bold values denote statistical significance at P < .05.
†Mann-Whitney U test. zChi-square test. xFisher exact test.
CV-high cohort had a higher proportion of patients experiencing severe AEs (n ¼ 24; 15.3%) compared with those in the IgE CVlow cohort (n ¼ 13; 8.3%); however, the difference was not statistically significant (P ¼ .057). Nevertheless, patients in the IgE CV-high cohort experienced more severe AEs than did patients in the IgE CV-low cohort (0.31 1.07 vs 0.10 0.34; P ¼ .047).
Negative binominal regression analysis showed that patients in the IgE CV-high cohort had a higher rate of severe AEs (RRadj ¼ 2.16; 95% CI, 1.08-4.32; P ¼ .029) (Figure 4, B). Similar to moderate-to-severe AEs, subgroup analyses indicated that patients in the IgE CV-high cohort with T2-low (RRadj ¼ 3.05; 95% CI, 1.17-7.97; P ¼ .023) or late-onset asthma (RRadj ¼ 2.66; 95% CI, 1.24-5.70; P ¼ .012) had a significantly higher rate of severe AEs. Results without adjusting for confounders are shown in the Online Repository (see Figure E1, B in this article's Online Repository at www.jaci-inpractice.org).
The predictive properties of IgE CV-high for AEs are presented in Tables E2 and E3 (in this article's Online Repository at www.jaci-inpractice.org). As a single predictor, IgE CV had a potential predictive value for AE with modest sensitivity (>70%) and a high negative predictive value (nearly or more than 90%) in patients with asthma in the T2-low, allergic, smoking, and well-controlled groups.
Furthermore, regardless of clinical history, FENO, and complete blood cell count, baseline IgE was not associated with future moderate-to-severe (Table E4, in this article's Online Repository at www.jaci-inpractice.org) or severe AEs (see Table E5 and Methods in this

article's Online Repository at www.jaci-inpractice.org). Similarly, mean IgE level was not associated with AEs during the following year (see Tables E6 and E7 and Methods in this article's Online Repository at www.jaciinpractice.org).

Mediation analyses of sputum IL-6 and IL-8 in the relationship of IgE CV with AEs

In the previous logistic regression models, we found that both sputum IL-6 and IL-8 were associated with IgE CV (Table III). However, whether sputum IL-6 and IL-8 mediated the relationship between IgE CV and AEs was unclear. Therefore, we used parallel multiple mediation models to examine whether the relationship between IgE CV and AEs was mediated by sputum IL-6 and/or IL-8 (Figure 2, A). As a result, the indirect effects (IEs) of IgE CV on moderate-to-severe (IE of severe to moderate AEs: point estimate of a1 b1 ¼ 0.085, bias-corrected [BC] 95% CI, 0.002-0.200) (Figure 2, B) and severe (IE of severe AEs: point estimate of a1 b1 ¼ 0.070, BC 95% CI, 0.0030.165) (Figure 2, C) AEs through sputum IL-6 were statistically significant. However, the indirect effects of IgE CV on moderateto-severe AEs and severe AEs through sputum IL-8 were not found, because the corresponding BC 95% CIs included zero. These findings indicate that sputum IL-6 mediated the effect of IgE CV on moderate-to-severe and severe AEs, whereas sputum IL-8 did not.

DISCUSSION

To the best of our knowledge, this is the first study to assess total lg (IgE) CV within a 1-month period to determine the IgE variability and explore its association with the future risk for AEs. Our study indicates that sputum IL-6 mediates the relationship between IgE CV and moderate-to-severe and severe AEs.

Compared with patients in the IgE CV-low cohort, those in the IgE CV-high cohort had worse asthma control, worse lung function presented as FEV1 and forced vital capacity, and higher ICS dosage, blood neutrophils, and IL-6 and IL-8 levels in the induced sputum. Furthermore, patients in the IgE CV-high cohort had a 1.88 higher rate of moderate-to-severe AEs than did patients in the IgE CV-low cohort during the follow-up year. Subgroup analyses indicated that increased rates of AEs were more pronounced in patients with T2-low, late-onset, and wellcontrolled asthma groups. In our study, sputum IL-6 levels mediated the effect of IgE CV on moderate-to-severe and severe AEs, whereas sputum IL-8 level did not. This study confirmed IgE variability as an easily obtained and clinically relevant measurement for AE, which suggests that serum IgE variability may be applied for asthma management and prognostic evaluation.

The serum total IgE concentration is influenced by multifaceted factors, such as age, sex, BMI, smoking status, family

FIGURE 4. Adjusted rate ratios (RRadj) for moderate-to-severe asthma exacerbations (A) and severe asthma exacerbations (B) in patients with IgE coefficient of variation-high (vs IgE coefficient of variation-low) for 1-year follow-up by negative binominal regression models. Adjusted for age, sex, body mass index, smoking status, atopic status, forced expiratory volume in 1 s % predicted, inhaled corticosteroid dosage, and baseline IgE level. Bold values denote statistical significance at $P < .05$.

history of asthma, age at asthma onset, and environmental factors (eg, allergen exposure).10,43-45 In people with asthma, young age, active smoking, male sex, high BMI, childhood-onset asthma, and asthma family history are positively associated with the level of serum total IgE.10,43-45 However, in our study, there were no differences in age, sex,

BMI, smoking status, age of asthma onset, and family history between cohorts. Previous studies showed that seasonal allergen exposure is associated with increased serum IgE concentrations in patients with seasonal allergies.46,47 Patients in the IgE CV-high cohort had lower proportions of either seasonal and/or perennial allergens than did patients in the IgE CV-low cohort. Nevertheless, the relationship between IgE CV and AEs was significant by adjusting patients' atopic status, which indicated that the allergens were not the cause of IgE CV-high with increased AEs.

In post-hoc analyses, patients with non-T2 high and late-onset asthma with greater IgE variability were at a significantly increased risk for experiencing AE. IgE is known for its roles in binding with antigens such as viral antigens or staphylococcal enterotoxins,48 both of which have important roles in the pathogenesis of non-T2 high and late-onset asthma. Further studies are needed to elucidate the correlation of IgE variability with viral or bacterial infection in non-T2 high or late-onset asthma in individuals who experienced AE. Interestingly, a positive relationship between the rates of AE and IgE variability irrespective of atopic status was observed in our study. IgE has a critical role in the pathogenesis of atopic asthma, even though several studies have shown that regardless of atopic status, allergic and nonallergic asthma are identical regarding bronchial mucosal cellular and molecular immunopathology.49-51 The clinical effectiveness of anti-IgE monoclonal antibodies such as omalizumab in nonatopic asthma lends weight to this view. Garcia et al52 conducted the first randomized controlled clinical trial to explore the effects of omalizumab in patients with nonallergic asthma. Omalizumab-treated patients had a decrease in AEs, which was consistent with a randomized controlled clinical trial showing that omalizumab therapy significantly improved lung function in nonatopic asthmatics.53 Moreover, a large real-world study, including 60 nonallergic patients with asthma, showed a significant reduction in severe AEs in omalizumab-treated patients.54 The modulatory effects of omalizumab could be attributed to reducing the expression of the high-affinity IgE receptor on plasmacytoid dendritic cells that enhance host antiviral immune responses to prevent AEs.52,54 Moreover, omalizumab therapy reduced the number of bronchial mucosal IgE-positive mast cells, which was associated with an improvement in lung function.53 Another hypothesis is that nonallergic asthma is not truly nonallergic, because it could be associated with elevated allergen-specific IgE antibodies in the airways owing to unidentified allergens.55 Future studies are needed to determine whether anti-IgE monoclonal antibodies could be used in allergic and nonallergic patients with asthma, with greater variability in serum total IgE levels, to reduce the future risk for AE.

Moreover, in our study, IgE variability was associated with sputum IL-6 and IL-8 levels. The mediation analysis indicated that sputum IL-6, not sputum IL-8, mediated the relationship between IgE CV and moderate-to-severe and severe AEs. IL-6 as a pleiotropic cytokine has an active role in the pathogenesis of asthma.56 A recently published study by Ghebre et al57 showed the association of high sputum IL-6 levels with increased risk for AEs. In addition, it confirmed that IL-6 affects the production of IgE, and vice versa. IgE can stimulate the autocrine production of IL-6 in human lung mast cells58; moreover, IL-6 participated in the induction of IgE synthesis.59 Similarly, in animal asthma models, the specific ablation of IL-6 in mouse macrophages reduced serum IgE levels.60 These findings might show that sputum IL-6 mediates the relationship between IgE CV and AEs, but this needs to be further studied in clinical trials. On the other hand, IL-8 is a potent neutrophil chemoattractant and has an important role in pathophysiologic mechanisms of asthma.61 Similar to IL-6, the release of IL-8 is associated with IgE. IgE receptor triggering leads to the

release of IL-8 from human lung.62 Although airway IL-8 levels are increased during AE, this would result from the response of airway epithelial cells to a wide variety of airway injuries, not by AE.63 Actually, it was supported by the study by Ghebre et al57 that sputum IL-8 levels cannot predict AE. These findings may be why IL-8 did not have a mediation effect on the relationship of IgE CV-high with AEs, but this would be need further study.

In our study, the lg (IgE) CV was associated with AEs. Moreover, lg (IgE) was poorly correlated with lg (IgE) CV (r ¼0.237; P < .001); also, it was not associated with moderate-to-severe or severe AEs during the follow-up year (Tables E6 and E7). This may be because the information obtained from the CV is additional to and independent of the mean. Similarly, the single serum total IgE concentration was not associated with AEs (Tables E4 and E5). This result was consistent with a recently published study conducted by Jackson et al,64 which showed that a single serum total IgE concentration was not associated with AEs in severe uncontrolled asthma. These findings further demonstrate the predictive power of IgE variability in relation to future AEs.

Strengths of this study include the prospective cohort study design in a real-world setting and the definition of IgE variability using a statistical method. Moreover, IgE variability is easy to obtain and practical for clinical use. However, there were some limitations associated with our study. We did not consider seasonal allergens in testing serum total IgE concentrations during the 1-month follow-up. However, there were no significant differences in IgE CV values either in seasonal (Figure E2, A) or monthly (Figure E2, B) distribution and no significant differences in patient proportions in seasonal (Figure E2, C) or monthly (Figure E2, D) distributions between cohorts. These results suggest that in our study, the samples had no impact on IgE CV. Also, measurement bias for estimating IgE CV may have resulted from the fact that only two observations of IgE during a 1-month period were used. To explore the impact of the number and period of observations on the IgE CV, another cohort of 114 patients with stable asthma, with three IgE levels within a 3month period, named the 3-month cohort (sample size ratio of the 1-month cohort to the 3-month cohort ¼ 3:1) was recruited. These patients were recruited from the China Center of Australasian Severe Asthma Network between June 2019 and October 2020. Patients in the 3-month cohort were tested three times for serum total IgE at baseline and after 1 and 3 months, respectively (see Table E8 in this article's Online Repository at www.jaci-inpractice.org). Furthermore, we compared the lg (IgE) CV of the 1-month cohort with the independent external 3-month cohort. There was no significant difference in the lg (IgE) CV (Table E8). Also, there was no significant difference in intraclass correlation coefficients between cohorts (Z ¼ 0.4; P ¼ .689) (see Table E9 in this article's Online Repository at www.jaci-inpractice.org), which indicated that the reliability of the two observations within 1 month of the 1-month cohort was not inferior to the three observations within 3 months of the 3-month cohort. Moreover, we believe that testing blood total serum IgE twice a month is more practical and acceptable than more tests within a longer period in clinical practice. Logarithm base 10 (IgE) CV obtained over a longer time should be performed in future studies. Finally, although we demonstrated the association between IgE variability and AEs, further investigation is needed to determine whether anti-IgE therapy can reduce the variability of IgE and prevent AEs.

CONCLUSION

We demonstrated that compared with patients with less IgE CV, those with greater IgE CV exhibited significantly worse asthma control, a higher intensity of asthma treatments, worse lung function, and more marked airway inflammation. Moreover, greater IgE variability was

an independent risk factor for future AEs. This study suggests that serum IgE-CV is an easy and practical measurement of AEs with clinical relevance. Future studies are needed to demonstrate whether IgE-CV, along with anti-IgE monoclonal antibody treatment, could be part of precision medicine and asthma management.

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