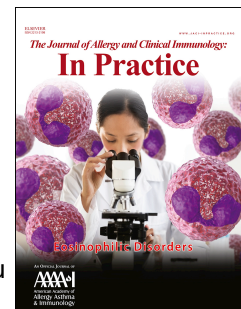


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TITLE PAGE

Total IgE variability is associated with future asthma exacerbations: a one-year prospective cohort study

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conducted the participant recruitment and participated in data analysis and
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ABSTRACT

Background: Few prospective studies have investigated the relationship between IgE variability and risk of 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 one-month) to obtain 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's median and followed up within a 12-month period during which, the association between IgE variability and AEs was explored using a negative binomial regression model.

Results: IgE CV levels obtained from 340 patients classified the 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% (Q1, Q3: 0.98%, 3.91%). IgE CV-high patients exhibited worse asthma control and lung function, more marked airway inflammation, and received more intensive medication use compared to IgE CV-low patients. IgE CV-high patients exhibited increased rates of moderate-to-severe (adjusted rate ratio [RR_{adj}]=2.88, 95% CI=[1.65, 5.03], $P<0.001$) and severe (RR_{adj}=2.16, 95% CI=[1.08, 4.32], $P=0.029$) AEs during the follow-up year compared to 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. Future studies are needed to investigate whether IgE variability can be used to guide precision medicine in asthma.

Highlights box:**1. What is already known about this topic?**

Immunoglobulin (Ig) E plays a critical role in the pathophysiology of both in allergic and non-allergic asthma. However, data on the relationship between IgE variability and asthma exacerbations are limited.

2. What does this article add to our knowledge?

IgE variability can be determined using the IgE coefficient of variation. Patients with asthma with higher IgE variability have an increased risk of future asthma exacerbations demonstrating the practical clinical utility of IgE variability.

3. How does this study impact current management guidelines?

Variability in total serum IgE levels is an easily obtained and practical measure for predicting asthma exacerbations that may be helpful for physicians to identify patients with asthma who are at risk.

Key words: asthma, IgE, variability, exacerbation, cohort study

Abbreviations list

ACQ: Asthma Control Questionnaire

ACT: asthma control test

AEs: asthma exacerbations

ASAN: Australasian Severe Asthma Network

ATS: American Thoracic Society

- 106 BDP: beclomethasone equivalents
- 107 BMI: body mass index
- 108 CI: confidence interval
- 109 CV: coefficient of variation
- 110 EA: eosinophilic asthma
- 111 ED: emergency department
- 112 ERS: European Respiratory Society
- 113 $F_{E}NO$: fractional exhaled nitric oxide
- 114 FEV_1 : forced expiratory volume in 1 second
- 115 FVC: forced vital capacity
- 116 GINA: Global Initiative for Asthma
- 117 ICS: inhaled corticosteroid
- 118 ICU: intensive care unit
- 119 Ig: immunoglobulin
- 120 IL: interleukin
- 121 IFN: interferon
- 122 Lg: logarithm base 10
- 123 LLD: lower limit of detection
- 124 MGA: mixed granulocytic asthma
- 125 NA: neutrophilic asthma
- 126 NEA: non-eosinophilic asthma
- 127 OR_{adj} : adjusted odds ratio

- 128 PGA: paucigranulocytic asthma
- 129 Q: quartile
- 130 RR_{adj} : adjusted rate ratio
- 131 SAWD: Severe Asthma Web-based Database
- 132 SD: standard deviation
- 133 SPT: skin prick test
- 134 TNF: tumor necrosis factor

INTRODUCTION

Asthma exacerbations (AEs) can have serious consequences that require hospitalization and can even be fatal, and are associated with significant healthcare costs^{1, 2}. AE occurs in the general population of asthmatics, and is not confined to patients with more severe diseases³. In 2019, approximately 15% of people with asthma required hospitalization in China⁴, and there are 500,000 hospitalizations per year due to AEs costing nearly \$5 billion annually in the USA⁵. To prevent exacerbations and their detrimental consequences, it is important to identify patients who are at risk.

Immunoglobulin (Ig) E is a characteristic feature of type I hypersensitivity and a key trigger for allergic airway inflammation in asthma⁶. Previous studies have reported its critical roles in the pathophysiology of allergic and non-allergic asthma⁷⁻⁹. Serum total IgE levels have exhibited correlations with asthma control, lung function, and asthma severity¹⁰⁻¹². However, evidence of the utility of the serum total IgE levels as a predictive biomarker for AE is limited. Semprin *et al.* 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 asthma¹³. Similarly, the Severe Asthma Research Program (SARP)-3 cohort identified an inverse relationship between baseline IgE levels and frequent of AEs, which could not be replicated in either the SARP-1 or SARP-2 cohorts¹⁴. These irreproducible results indicate that a single measured IgE value is an insufficient predictor of future AEs.

Recently, several studies have discovered evidence of intrasubject variability in total IgE levels as a function of time, which may be related to AEs¹⁵⁻¹⁶. In a retrospective cohort study, Tanaka *et al.* observed that patients with increased levels of serum total IgE experienced more AEs than did patients with decreased or unchanged IgE levels¹⁷. However, IgE variability in that study was defined arbitrarily by total IgE at the time of the study minus total IgE detected 10 years earlier¹⁷. There

is no gold standard method for assessing the variability of IgE levels. Previous studies have recommended that the definition of peak expiratory flow variability in patients with asthma should be based on the coefficient of variation (CV)¹⁸⁻¹⁹. CV represents the ratio of the standard deviation to the mean¹⁹, and is a useful statistic for comparing the degree of variation from one data series to another and thus 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 have been previously presented as an abstract²⁰.

METHODS

Study design and patients

This was a real-world, one-year prospective cohort study designed to explore the relationship of IgE variability with AEs. This study comprised two parts. In the enrollment phase, patients were followed for one-month to determine the base 10 log-transformed (lg) serum total IgE CV. The median of serum total lg (IgE) CV in our patients was used as the cut-off value to divide the patients into two cohorts: lg (IgE) CV-high or lg (IgE) CV-low cohort (one-month cohort). Then, these patients were followed up for 12-months (face-to-face visits or telephone calls, if unavailable) to assess the occurrence of AEs (Figure 1). AE-related details, including dates of occurrence, treatments and the 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 (ASAN)²¹, some of which has been previously published²²⁻²⁴. 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 ChiCTR-OOC-16009529 (<http://www.chictr.org.cn>).

Patients with stable asthma aged ≥ 18 years 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 obstruction²⁵. Variable airflow obstruction was confirmed by evidence of either airway hyper-responsiveness or bronchodilator responsiveness with a more than 12% and 200mL increase from the baseline in forced expiratory volume in one second (FEV₁)²⁵. Stable asthma was defined as exacerbation-free, no respiratory infection, or no change in maintenance therapy in

the preceding four weeks²⁶. Patients who were pregnant, breastfeeding or had other chronic unstable diseases were excluded.

As a real-world study, indications for patient treatment were based on the GINA recommendations²⁵. Step-up 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, atopic status²²⁻²⁴. Asthma control and quality of life were assessed using the asthma control test (ACT) score and asthma quality of life questionnaire (AQLQ), respectively, which have been validated in the Chinese population^{27, 28}. Asthma control was determined by ACT score, in which scores in the range of 20–25 classified as well-controlled asthma, 16–19 as partial-controlled and 5–15 as uncontrolled asthma. Atopic status was determined by at least one positive skin prick test (SPT) for common local seasonal and perennial allergens as previously described^{25, 29}. In our study, seasonal allergens³⁰ comprised mixed tree pollen (birch, London plane) and mixed grass pollen (ragweed, humulus, *Artemisia annua*), while perennial allergens³¹ included house dust mites, dog and cat hair, and alternaria. The fraction of exhaled nitric oxide³² (F_ENO, NIOX analyzer, Aerocrine, Solna, Sweden) and spirometry³³ (MedGraphics Corp., St. Paul, MN, USA) were measured based on the American Thoracic Society/European Respiratory Society (ATS/ERS) recommendations. In addition, all subjects underwent blood sampling and sputum induction.

Blood processing

Fasting venous blood samples, either in ethylenediamine tetra acetic 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 Immage 800 immunoassay analyzer, Beckman Coulter Inc., USA). All patients underwent two serum total IgE tests within a month: at screening (baseline IgE) and one-month (29 to 31 days)³⁴ later. The minimum detectable level of total IgE was 5.0 IU/mL.

Sputum processing and inflammatory cytokines testing

Sputum induction and processing were performed based on a protocol described in our previous published study³⁵. Briefly, sputum was induced using either 4.5% hypertonic saline or saline atomized with an ultrasonic nebulizer (Cumulus, HEYER Medical AG, Germany) in subjects with FEV₁% predicted $\geq 40\%$ or FEV₁% predicted $< 40\%$. Sputum plugs (100 μ L) were processed using 400 μ L dithiothreitol (DTT) and 400 μ L phosphate-buffered saline. Cytospins were prepared using a centrifugation-smear (CYTOPRO 7620, WESCOR®, INC, LOGAN, USA), while stained (May-Grunwald Giemsa), and a differential cell counts were obtained from 400 non-squamous cells. Differential cell counts were performed by well-trained researchers from the both University of Newcastle, NSW, Australia, and West China Hospital, China.

The sputum supernatant was aspirated and frozen at -80°C for testing interferon (IFN)- γ (lower limit of detection [LLD], 0.8 pg/mL), interleukin (IL)-1 β (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 tumor necrosis factor (TNF)- α (LLD, 0.8 pg/mL) by Luminex-based MILLIPLEX® MAP Human Cytokine/Chemokine Magnetic bead Panel Kit (EMD Millipore Corporation, Billerica, MA, USA). Spiking experiments of cytokines in sputum supernatants showed that recovery ranged from 70% to 130% for all detectable analyses³⁶.

Asthma phenotypes

Patients were classified into four inflammatory phenotypes as follows³⁷: eosinophilic asthma (EA, induced sputum eosinophils $\geq 3\%$ alone and/or blood

eosinophils $\geq 0.3 \times 10^9$ cells/L), neutrophilic asthma (NA, neutrophils $\geq 61\%$ and eosinophils $< 3\%$ in induced sputum), mixed granulocytic asthma (MGA, eosinophils $> 3\%$ and neutrophils $> 61\%$ in induced sputum), and paucigranulocytic asthma (PGA, neutrophils $< 61\%$ and eosinophils $< 3\%$ in induced sputum). Allergic asthma was defined as positive reaction to common allergens using SPT^{25, 29}. Late-onset asthma was defined as asthma onset of ≥ 12 years old³⁸. Type (T) 2-high asthma was defined as meeting two of the following three criteria: a total IgE level of more than 100 IU/mL, eosinophil count $\geq 0.14 \times 10^9$ cells/L, and $F_E NO \geq 30$ ppb^{39, 40}.

Definition of total IgE variability

Serum total IgE values were base-10 log-transformed to obtain a normal distribution. 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.

$$CV = \frac{\sqrt{(x_1 - \bar{x})^2 + (x_2 - \bar{x})^2}}{\bar{x}}$$

where x_1 = lg (IgE) at baseline, x_2 = lg (IgE) after one month, $\bar{x} = (x_1 + x_2)/2$.

Primary and secondary outcomes

The primary and secondary outcomes were moderate-to-severe AEs and severe AEs during the follow-up period, respectively. AE was defined based on the ATS/ERS statement⁴¹. Therefore, moderate AE was defined as deterioration in symptoms and in lung function, and increased rescue bronchodilator use, with a duration of ≥ 2 days, but not be severe enough to warrant systemic corticosteroid use⁴¹. 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 due to asthma exacerbation, requiring systemic corticosteroids for at least 3 days⁴¹. For consistency, courses of corticosteroids separated by one week or more were treated as separate severe exacerbations⁴¹.

Statistical analysis

Categorical variables were summarized as frequencies and proportions, and continuous variables were expressed as mean (standard deviation, SD) or median (interquartile range). If possible, all continuous data were transformed into a normal distribution. The difference between cohorts for each variable was evaluated using a Student's t test or Mann-Whitney U test for continuous variables, and the Chi-square test or Fisher's exact test for categorical variables, as appropriate.

Logistic regression analysis was used to assess the associations between single sputum cytokines and IgE CV with or without adjusting for age, sex, body mass index (BMI), smoking status, FEV₁% predicted, 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 due to the potential clinical significance of baseline IgE and mean IgE levels of baseline and one-month later for AEs prediction. (Online Repository Text)

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 4A). Mediation hypotheses were tested using bias-corrected bootstrapping with 10,000 samples to calculate confidence intervals (95%) with the PROCESS macro (version 3.3) for SPSS 22.0 (SPSS, Chicago, IL, USA) using ordinary least squares regression to estimate variables on the left side of the model equations⁴². Significance was achieved when an indirect effect ($a \times b$) was observed and zero was not included in the confidence intervals, instead of individual

309 tests of the paths of the model (c, a, and b)⁴². Detailed information is provided in the
310 Online Repository Text.

311 Statistical analysis was performed using IBM SPSS 22.0 software (version 22.0;
312 SPSS, Chicago, IL, USA). All tests were two-tailed, and a *P*-value less than 0.05 was
313 considered statistically significant.

314

RESULTS

Demographic and clinical characteristics

Three hundred and forty-seven patients were included in the baseline testing. During one-month follow-up, seven patients were excluded due to experience AE (n=3), inability to make contact (n=1), or refusal to participate (n=3) without IgE CV. Finally, IgE CV levels were successfully obtained from 340 patients who were classified into two groups (n=170 for the IgE CV-high and IgE CV-low groups, respectively) based on the median of serum total IgE CV of 2.12% (Q1, Q3: 0.98%, 3.91%). Then, these two cohorts were prospectively followed up for 12 months, in which 27 patients were excluded due to inability to make contact (n=12) or refusal to participate (n=15). Therefore, 313 patients (IgE CV-high cohort, n=157; IgE CV-low cohort, n=156) were included in the final analysis (Figure 1).

Compared with the patients in IgE CV-low cohort, the patients in IgE CV-high cohort had worse asthma control (IgE CV-high vs.-low: ACT scores, 19.00 [15.00, 22.00] vs. 20.00 [16.75, 22.25], $P=0.012$), worse lung function presented as lower capacity of FEV₁ (2.07 ± 0.78 vs. 2.21 ± 0.76 L, $P=0.047$) and FVC (3.03 [2.42, 3.60] vs. 3.27 [2.71, 3.73] L, $P=0.035$), and received more intensive ICS medication (beclometasone dipropionate [BDP] equivalent: 608.28 ± 510.65 vs. 539.36 ± 482.93 µg/day, $P=0.019$) (Table 1).

In terms of asthma phenotypes, IgE CV-high cohort had a lower proportion of patients with T2-high (IgE CV-high vs. low: 35.3% vs. 64.7%, $P<0.001$) and allergic (48.2% vs. 67.6%, $P<0.001$) asthma but a higher proportion of late-onset asthma (87.6% vs. 79.4%, $P=0.041$), than IgE CV-low cohort. However, there were no differences between the two cohorts in asthma inflammatory phenotypes ($P=0.775$) (Table 1).

Compared with the patients in IgE CV-low cohort, the patients in IgE CV-high cohort had lower levels of total IgE both at baseline (IgE CV-high vs.-low: 88.34

[32.56, 221.03] vs. 207.75 [82.57, 463.39] IU/mL, $P<0.001$) and one-month later (80.61 [32.71, 171.75] vs. 202.87 [79.91, 444.78] IU/mL, $P<0.001$). Moreover, the patients in IgE CV-high cohort had lower proportions of either sensitization of seasonal or perennial allergens than the patients in IgE CV-low cohort (both $P<0.05$) (Table 1).

Local and systemic inflammation

Patients in IgE CV-high cohort had significant airway inflammation characterized by higher levels of sputum IL-6 and IL-8 compared with the patients in IgE CV-low cohort (IgE CV-high vs. -low: 20.84 [8.92, 51.88] vs. 13.66 [5.20, 32.56] pg/mL, $P=0.019$; 1337.00 [808.83, 3106.00] vs. 1108.00 [579.67, 2218.00] pg/mL, $P=0.028$; respectively) (Figure 2). However, there were no significant differences either in counts or percentages of sputum granulocytes (including total cells, neutrophils, eosinophils, macrophages, and lymphocytes) and F_{ENO} (all $P>0.05$) between the IgE CV-high and IgE CV-low cohorts (Table 2).

Furthermore, we compared the inflammatory cells in the peripheral blood between the two cohorts. Patients in IgE CV-high cohort had more neutrophils (IgE CV-high vs. -low: $3.86 [2.87, 4.92] \times 10^9$ cells/L vs. $3.38 [2.82, 4.15] \times 10^9$ cells/L, $P=0.027$) and lower percentage of eosinophils ($3.50 [1.73, 6.28] \%$ vs. $4.40 [2.34, 6.87] \%$, $P=0.036$) in the blood than the patients in IgE CV-low cohort (Table 2).

The associations of IgE variability with airway inflammatory profiles

We explored the associations between sputum inflammatory cytokines and IgE CV using a logistic regression model by without adjusting (Table E1) and adjusting (Table 3) confounders including age, sex, BMI, smoking status, FEV₁% predictive, ICS dosage, and baseline IgE concentrations. The levels of IL-6 (adjusted odds ratio [OR_{adj}]=2.01, 95% CI= [1.10–3.66], $P=0.022$) and IL-8 (OR_{adj}=2.41, 95% CI= [1.08–5.39], $P=0.032$) in induced sputum were significantly associated with increased IgE variability. The result of the level of IL-1 β and IgE CV, although suggestive of an

association, did not achieve statistical significance ($OR_{adj}=1.62$, 95% CI= [0.96–2.74], $P=0.074$).

Associations of IgE variability with asthma exacerbations by negative binomial regression analyses

Moderate-to-severe AEs

Fifty patients (16.0%) experienced moderate-to-severe AEs ($n=170$) in the one-year follow-up. Compared with patients in the IgE CV-low cohort, more moderate-to-severe AEs occurred in the patients in IgE CV-high cohort than the patients in IgE CV-low cohort ($n=17$, 10.9% vs. $n=33$, 21.0%; $P=0.015$). Also, frequencies of moderate-to-severe AE were greater in the patients in IgE CV-high cohort than the patients in IgE CV-low cohort (0.48 ± 1.26 vs. 0.14 ± 0.45 , respectively) ($P=0.006$) (Table 4).

Furthermore, the negative binomial regression analysis showed that IgE CV variability was significantly associated with moderate-to-severe AEs (Figure 3). The rate of moderate-to-severe AEs was significantly higher for the patients in IgE CV-high cohort compared with the patients in IgE CV-low cohort (adjusted rate ratio [RR_{adj}]=2.88, 95% CI=[1.65–5.03], $P<0.001$) after adjusting for age, sex, BMI, smoking status, atopic status, FEV₁% predicted, ICS dosage and baseline IgE level (Figure 3A).

Subgroup analyses indicated that the patients in IgE-CV high cohort with T2-low ($RR_{adj}=4.13$, 95% CI=[1.95–8.78], $P<0.001$), late-onset asthma ($RR_{adj}=2.26$, 95% CI=[1.78–5.97], $P<0.001$), non-smoking asthma ($RR_{adj}=2.45$, 95% CI=[1.28–4.72], $P=0.007$), or well-controlled asthma ($RR_{adj}=3.63$, 95%CI=[1.23–10.81], $P=0.020$), had experienced significantly more moderate-to-severe AEs than those in the IgE CV-low cohort. Surprisingly, the atopic status did not affect the association of IgE variability with AEs since patients with both allergic ($RR_{adj}=2.93$, 95% CI=[1.35–6.35], $P=0.006$) and non-allergic ($RR_{adj}=2.65$, 95% CI=[1.02–6.92], $P=0.046$) asthma

in the IgE CV-high cohort had an increased risk of experiencing moderate-to-severe AEs when the IgE CV-low cohort was used as the reference (Figure 3A).

Severe AEs

Thirty-seven patients (11.8%) experienced severe AEs (n=64) in the one-year follow-up (Table 4). The patients in IgE CV-high cohort had a higher proportion of patients experiencing severe AE (n=24, 15.3%) compared with the patients in IgE CV-low cohort (n=13, 8.3%); however, the difference was not statistically significant ($P=0.057$). Nevertheless, the patients in IgE CV-high cohort had experienced more severe AEs than the patients in IgE CV-low cohort (0.31 ± 1.07 vs. 0.10 ± 0.34 , $P=0.047$).

Negative binominal regression analysis showed that patients in the IgE CV-high cohort had a higher rate of severe AEs ($RR_{adj}=2.16$, 95% CI=[1.08–4.32], $P=0.029$) (Figure 3 B). Similar to moderate-to-severe AEs, the subgroup analyses indicated that the patients in IgE CV-high cohort with T2-low ($RR_{adj}=3.05$, 95% CI=[1.17–7.97], $P=0.023$) or late-onset asthma ($RR_{adj}=2.66$, 95% CI=[1.24–5.70], $P=0.012$) had significantly a higher rate of severe AEs. The results without adjusting for confounders are shown in the Online Repository Text (Figure E1 B).

The predictive properties of IgE CV-high for AEs are presented in the Online Repository Text (Table E2–3). As a single predictor, IgE CV had a potential predictive value for AE with modest sensitivity (>70%) and high negative predictive value (nearly or more than 90%) in the patients with asthma in the T2-low, allergic, smoking, and well-controlled groups.

Furthermore, regardless of clinical history, F_{ENO} , and complete blood cell count, baseline IgE was not associated with future moderate-to-severe (Table E4) or severe AEs (Online Repository Text and Table E5). Similarly, mean IgE level was not associated with AEs during the following year (Online Repository Text and Table E6–7).

Mediation analyses of sputum IL6 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 3). 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. As a result, the indirect effects of IgE CV on moderate-to-severe (IE of severe-moderate AEs: point estimate of $a_1 \times b_1 = 0.085$, BC 95% CI [0.002–0.200], Figure 4B) and severe (IE of severe AEs: point estimate of $a_1 \times b_1 = 0.070$, BC 95% CI [0.003–0.165], Figure 4C) AEs through sputum IL-6 were statistically significant. However, neither the indirect effects of IgE CV on moderate-to-severe AEs and severe AEs through sputum IL-8 were found, as 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, while sputum IL-8 did not.

437 DISCUSSION

438 To the best of our knowledge, this is the first study to assess total Ig (IgE) CV
 439 within a one-month period to determine the IgE variability and to explore its
 440 association with the future risk of AEs. Our study is the first to indicate that sputum
 441 IL-6 mediates the relationship between IgE CV and moderate-to-severe and severe
 442 AEs.

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

455 The serum total IgE concentration is influenced by multifaceted factors, such as
 456 age, sex, BMI, smoking status, family history of asthma, age at asthma onset, and
 457 environmental factors (e.g., allergen exposure)^{10, 43-45}. In people with asthma, being
 458 young age, active smoking, male sex, high BMI, childhood-onset asthma, and asthma
 459 family history are positively associated with the level of serum total IgE^{10, 43-45}.
 460 However, in our study, there were no difference in age, sex, BMI, smoking status, age
 461 of asthma onset, and family history between the two cohorts. Previous studies have
 462 shown that seasonal allergen exposure is associated with increased serum IgE
 463 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 patients in the IgE CV-low cohort. Nevertheless, the relationship between IgE CV and AEs was still 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 of experiencing AE. IgE is known for its roles in binding with antigens such as viral antigens or staphylococcal enterotoxins⁴⁸, both of which play 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 plays a critical role in the pathogenesis of atopic asthma, despite the fact that several studies have shown that, regardless of the atopic status, allergic and non-allergic asthma are identical regarding their bronchial mucosal cellular and molecular immunopathology⁴⁹⁻⁵¹. The clinical effectiveness of anti-IgE monoclonal antibodies, such as omalizumab, in non-atopic asthma lends weight to this view. Garcia G *et al.* conducted the first randomized controlled clinical trial to explore the effects of omalizumab in patients with non-allergic asthma⁵². Omalizumab-treated patients presented a decrease in AEs⁵², which was consistent with a randomized controlled clinical trial showing that omalizumab therapy significantly improved the lung function in non-atopic asthmatics⁵³. Moreover, a large real-world study, including 60 non-allergic patients with asthma, showed a significant reduction in severe AEs in omalizumab-treated patients⁵⁴. The modulatory effects of omalizumab could be attributed to reduce 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 also reduced the number of bronchial mucosal IgE⁺ mast cells that was

associated with the improvement of lung function⁵³. Another hypothesis is that non-allergic asthma is not truly non-allergic, as it could be associated with elevated allergen specific IgE antibodies in the airways due to unidentified allergens⁵⁵. Future studies are needed to determine whether anti-IgE monoclonal antibodies could be used in allergic and non-allergic patients with asthma, with greater variability in serum total IgE levels, to reduce the future risk of 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 plays an active role in the pathogenesis of asthma⁵⁶. A recently published study by Ghebre *et al.* has shown the association of high sputum IL-6 levels with increased risk of AEs⁵⁷. In addition, it has 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, meanwhile IL-6 participated in the induction of IgE synthesis⁵⁹. Similarly, in animal asthma models, the specific ablation of IL-6 in mouse macrophages reduced serum IgE levels⁶⁰. These findings might explain that sputum IL-6 mediate the relationship between IgE CV and AEs, but it needs to be further studied in clinical trials. On the other hand, IL-8 is the potent neutrophil chemoattractant and plays an important role in the patho-physiological mechanisms of asthma⁶¹. 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 lung⁶². 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 AE⁶³. Actually, it was somewhat supported by the study by Ghebre *et al.* that sputum IL-8 levels can't predict AE⁵⁷. These findings may be the reasons why IL-8 did not have mediation effect on the relationship of IgE CV-high with AEs, but it would be needed to be studied in future.

In our study, the lg (IgE) CV was associated with AEs. Moreover, lg (IgE) was poorly correlated with lg (IgE) CV ($r=-0.237$, $P<0.001$); meanwhile, it was not

associated with moderate-to-severe or severe AEs during the follow-up year (Table E6–7). This may be due to the fact that 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 (Table E4–5). This result was consistent with a recently published study conducted by Jackson *et al.*, which showed that single serum total IgE concentration was not associated with AEs in severe, uncontrolled asthma⁶⁴. These findings further demonstrate the predictive power of IgE variability in relation to future AEs.

The 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, the IgE variability is easy to obtain and practical for clinical use. However, there were some limitations associated with our study. We did not consider the seasonal allergen in testing serum total IgE concentrations during the one-month follow-up period. 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) and monthly (Figure E2 D) distributions between the two cohorts. These results suggest that, in our study, the samples taken 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 one-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 3-month period, named the 3-month cohort (sample size ratio of the one-month cohort to the three-month cohort=3/1) was recruited; these patients were recruited from the China Center of ASAN between June 2019 and October 2020. Patients in the 3-month cohort were tested three times for serum total IgE at baseline and after one month and 3 months, respectively (Table E8). Furthermore, we compared the lg (IgE) CV of the one-month cohort to the independent external three-month cohort. There was no significant difference in the lg

(IgE) CV (Table E8). Also, there was no significant difference in the intraclass correlation coefficients between the two cohorts ($Z=0.4$, $P=0.689$) (Table E9), which indicated that the reliability of the two observations within one month of the one-month cohort was not inferior to the three observations within three months of the three-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. Lg (IgE) CV obtained over a longer period of 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 to those with less IgE CV, patients 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 asthma exacerbations 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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578 **CONFLICT OF INTEREST STATEMENT**

579 On behalf of all authors, the corresponding author states that there is no conflict
580 of interest.

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Tables

Table 1. Demographic and clinical characteristics of patients grouped by total Ig (IgE) CV within one-month

Characteristics	IgE CV-high	IgE CV-low	$t/Z/\chi^2$	P-value
n	170	170		
Age, years, median (Q1, Q3)	44.33 (34.89, 55.55)	43.57 (34.93, 53.79)	-0.713	0.476 ^{††}
Female, n (%)	108 (63.5)	109 (64.1)	0.013	0.910 ^{†††}
BMI, kg/m ² , median (Q1, Q3)	23.10 (20.70, 25.10)	23.22 (21.22, 25.25)	-0.539	0.590 ^{††}
Smoking, pack-years, mean (SD)	3.1 (8.9)	2.6 (8.6)	-1.811	0.070 ^{††}
Atopic status, n (%)	82 (48.2)	115 (67.6)	13.143	0.001 ^{†††}
Seasonal allergens\$, n (%)	21 (12.4)	36 (21.2)	4.742	0.029 ^{†††}
Perennial allergens\$\$, n (%)	61 (35.9)	79 (46.5)	3.934	0.047 ^{†††}
Seasonal and perennial allergens, n (%)	9 (5.3)	22 (12.9)	5.999	0.014 ^{†††}
Family history, n (%)	61 (35.9)	66 (38.8)	0.044	0.835 ^{†††}
Age of onset, years, median (Q1, Q3)	34.00 (22.00, 46.00)	32.00 (20.00, 44.00)	1.330	0.184 ^{†††}
ACT score, median (Q1, Q3)	19.00 (15.00, 22.00)	20.00 (16.75, 22.25)	-2.525	0.012 ^{††}
AQLQ score, median (Q1, Q3)	5.72 (5.00, 6.35)	5.90 (5.35, 6.37)	-1.732	0.083 ^{††}
HADS-A, median (Q1, Q3)	2 (0, 4)	1 (0, 3)	-1.548	0.122 ^{††}
HADS-D, median (Q1, Q3)	1 (0, 3)	1 (0, 4)	-1.707	0.088 ^{††}
Sinusitis, n (%)	79 (46.5)	85 (50.0)	0.384	0.535 ^{†††}
Medications				

ICS dosage, BDP equivalent, µg/d, mean (SD)	608.28 (510.65)	539.36 (482.93)	-2.341	0.019 ††
ICS/LABA, n (%)	95 (55.9)	100 (58.8)	0.301	0.583†††
LTRA, n (%)	154 (90.6)	150 (88.2)	0.497	0.481†††
OCS, n (%)	7 (4.1)	5 (2.9)	0.346	0.557†††
GINA steps 4–5, n (%)	36 (21.2)	44 (25.9)	1.046	0.306†††
Spirometry				
FEV ₁ , L, mean (SD)	2.06 (0.78)	2.21 (0.76)	-1.988	0.047 ††
FEV ₁ , % predicted, median (Q1, Q3)	72.00 (56.00, 88.00)	73.00 (29.50, 89.00)	-0.780	0.435††
FVC, L, median (Q1, Q3)	3.03 (2.42, 3.60)	3.27 (2.71, 3.73)	-2.107	0.035 ††
FEV ₁ /FVC, %, mean (SD)	65.14 (13.74)	66.55 (14.05)	0.553	0.349†
Asthma phenotypes				
Inflammatory phenotypes#, EA/NA/PGA, n (%)	58 (42.6)/25 (18.4)/53 (39.0)	56 (44.4)/18 (14.3)/52 (41.3)	0.510	0.775†††
Late-onset/early-onset asthma, n (%)	149 (87.6)/21 (22.4)	135 (79.4)/35 (10.6)	4.190	0.041 †††
T2-high/non-T2 high asthma, n (%)	60 (35.3)/ 110 (64.7)	102 (60.0)/68 (40.0)	21.392	<0.001 †††
Allergic/non-allergic asthma, n (%)	76 (44.7)/94 (55.3)	115 (67.6)/55 (32.4)	13.362	<0.001 †††
Lg (IgE) CV, %, median (Q1, Q3)	3.90 (2.83, 6.19)	0.98 (0.39, 1.43)	-15.927	<0.001 ††
Serum total IgE at screening, IU/mL, median (Q1, Q3)	88.34 (32.56, 221.03)	207.75 (82.57, 463.39)	-4.968	<0.001 ††
Serum total IgE after one-month later, IU/mL, median (Q1, Q3)	80.61 (32.71, 171.75)	202.87 (79.9, 444.78)	-5.578	<0.001 ††
ACT, Asthma Control Test; AQLQ, Asthma Quality of Life Questionnaire; BDP, beclomethasone equivalents; BMI, body mass index; CV, coefficient of variation; EA,				

eosinophilic asthma; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; GINA, Global Initiative for Asthma; ICS, inhaled corticosteroid; ICS/LABA, inhaled corticosteroid with long-acting beta-agonist; Ig, immunoglobulin; lg, logarithm base 10; LTRA, leukotriene receptor antagonist; NA, neutrophilic asthma; OCS, oral corticosteroid; Q1, first quartile; Q3, third quartile; PGA: paucigranulocytic asthma; SD, standard deviation; T2, type 2.

Mixed granulocytic asthma was not analyzed since only one patient was identified as having granulocytic asthma; eosinophilic asthma was defined as sputum eosinophils $\geq 3\%$ alone and/or blood eosinophils $\geq 0.3 \times 10^9$ cells/L; neutrophilic asthma was defined as sputum neutrophil count $\geq 61\%$ and eosinophil count $< 3\%$; paucigranulocytic asthma was defined as sputum neutrophil count $< 61\%$ and eosinophil count $< 3\%$.

\$ Seasonal allergens comprised mixed tree pollen and mixed grass pollen; \$\$ Perennial allergens comprised house dust mites, dog and cat hair, alternaria.

Statistical analyses were performed by † Student's t test, †† Mann-Whitney U test, ††† Chi-square test.

Bold values denote statistical significance at $P < 0.05$.

Table 2. Local and systemic inflammation in patients grouped by total Ig (IgE) variability

Variables	IgE CV-high	IgE CV-low	t/Z	P-value
n	170	170		
F _E NO, ppb, median (Q1, Q3)	28.50 (16.75, 66.0)	40.00 (22.75, 71.00)	-1.528	0.127††
Sputum				
Total cell count, ×10 ⁴ /mL, median (Q1, Q3)	36.00 (19.00, 66.2)	40.50 (16.25, 68.00)	-0.642	0.521††
Neutrophils				
×10 ⁴ /mL, median (Q1, Q3)	9.37 (2.41, 31.60)	8.02 (2.86, 27.45)	-0.098	0.922††
%, median (Q1, Q3)	37.38 (15.38, 59.06)	28.25 (12.05, 53.88)	-1.360	0.174†
Macrophages				
×10 ⁴ /mL, median (Q1, Q3)	14.06 (3.59, 26.54)	15.40 (6.01, 30.33)	-0.709	0.478††
%, median (Q1, Q3)	52.13 (18.81, 76.06)	62.25 (28.94, 82.56)	-1.796	0.073††
Eosinophils				
×10 ⁴ /mL, median (Q1, Q3)	0.06 (0.00, 0.67)	0.04 (0.00, 0.46)	-0.119	0.905††
%, median (Q1, Q3)	0.25 (0.00, 3.00)	0.25 (0.00, 1.19)	-0.789	0.430††
Lymphocytes				
×10 ⁴ /mL, median (Q1, Q3)	0.23 (0.00, 0.54)	0.25 (0.04, 0.48)	-0.468	0.640††
%, median (Q1, Q3)	0.50 (0.25, 1.25)	0.50 (0.25, 1.75)	-0.363	0.716††
Blood				
Neutrophils				
×10 ⁹ /L, median (Q1, Q3)	3.86 (2.87, 4.92)	3.38 (2.82, 4.15)	-2.210	0.027††
%, median (Q1, Q3)	61.1 (54.1, 66.1)	59.1 (53.2, 63.6)	-1.871	0.061††
Lymphocytes				
×10 ⁹ /L, median (Q1, Q3)	1.72 (1.45, 2.17)	1.72 (1.36, 2.05)	-0.996	0.319††
%, mean (SD)	28.65 (7.23)	28.9 (6.71)	-0.367	0.714†
Monocytes				
×10 ⁹ /L, median	0.36 (0.27, 0.49)	0.33 (0.28, 0.43)	-1.551	0.121††

(Q1, Q3)				
%, median (Q1, Q3)	5.81 (4.88, 7.04)	5.72 (4.87, 6.96)	-0.605	0.545 ^{††}
Eosinophils				
$\times 10^9/L$, median (Q1, Q3)	0.20 (0.10, 0.40)	0.25 (0.14, 0.42)	-1.312	0.190 ^{††}
%, median (Q1, Q3)	3.50 (1.73, 6.28)	4.40 (2.34, 6.87)	-2.095	0.036^{††}
Basophils				
$\times 10^9/L$, median (Q1, Q3)	0.04 (0.02, 0.06)	0.04 (0.02, 0.05)	-0.565	0.572 ^{††}
%, median (Q1, Q3)	0.54 (0.37, 0.82)	0.59 (0.41, 0.83)	-0.571	0.568 ^{††}
CV, coefficient of variation; F_ENO, fractional exhaled nitric oxide; Ig, immunoglobulin; lg, logarithm base 10; Q1, first quartile; Q3, third quartile; SD, standard deviation. Statistical analyses were performed by [†] Student's t test, ^{††} Mann-Whitney U test. Bold values denote statistical significance at the $P < 0.05$.				

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Table 3. Logistic regressions exploring associations of sputum cytokine with total Ig (IgE) CV-high cohort

Variables #	OR _{adj} (95% CI) *	P-value
IFN- γ , pg/mL	0.16 (0.01–5.19)	0.303
TNF- α , pg/mL	1.37 (0.73–2.59)	0.330
IL-4, pg/mL	0.86 (0.58–1.28)	0.465
IL-5, pg/mL	1.27 (0.56–2.88)	0.572
IL-1 β , pg/mL	1.62 (0.96–2.74)	0.074
IL-6, pg/mL	2.01 (1.10–3.66)	0.022
IL-8, pg/mL	2.41 (1.08–5.39)	0.032
IL-13, pg/mL	1.35 (0.46–3.98)	0.590
* Logistic regressions exploring the associations of sputum cytokine (independent variable) and total Ig (IgE) CV-high cohort (dependent variable) after adjusting for confounders, including sex, age, BMI, smoking status, FEV₁% predictive, ICS dosage and baseline total IgE level. BMI, body mass index; CI, confidence interval; CV, coefficient of variation; IFN, interferon; IL, interleukin; Ig, immunoglobulin; lg, logarithm base 10; OR_{adj}, adjusted odds ratio; TNF, tumor necrosis factor. # Sputum cytokine values have been base-10 log-transformed. Bold values denote statistical significance at $P < 0.05$.		

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Table 4: Asthma exacerbations in the follow-up year grouped by lg (IgE) CV

Outcomes	IgE CV-high	IgE CV-low	$Z/\chi^2/F$	P-value
n	157	156		
Moderate to severe AE				
n (%)	33 (21.0)	17 (10.9)	5.972	0.015 †††
Mean (SD)	0.48 (1.26)	0.14 (0.45)	-2.723	0.006 ††
Severe AE				
n (%)	24 (15.3)	13 (8.3)	3.629	0.057†††
Mean (SD)	0.31 (1.07)	0.10 (0.34)	1.987	0.047 ††
ED visits				
n (%)	6 (3.8)	2 (1.3)		0.283††††
Mean (SD)	0.11 (0.68)	0.01 (0.11)	-1.692	0.091††
Hospitalization				
n (%)	11 (7.0)	7 (4.5)	0.916	0.338†††
Mean (SD)	0.10 (0.39)	0.05 (0.25)	-0.972	0.331††
AE: asthma exacerbation; CV: coefficient of variation; ED: emergency department; Ig, immunoglobulin; lg, logarithm base 10; SD: standard deviation. Statistical analyses were performed by †† Mann-Whitney U test, ††† Chi-square test, †††† Fisher's exact test. Bold values denote statistical significance at the $P<0.05$.				

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Figure Legends

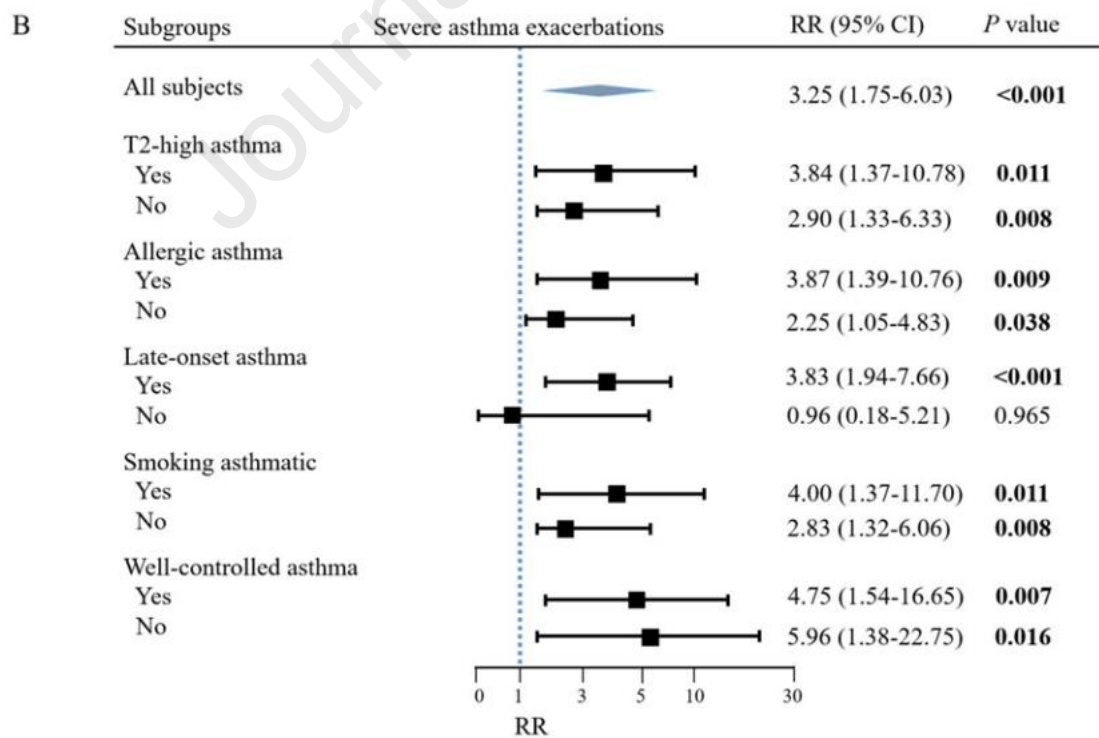
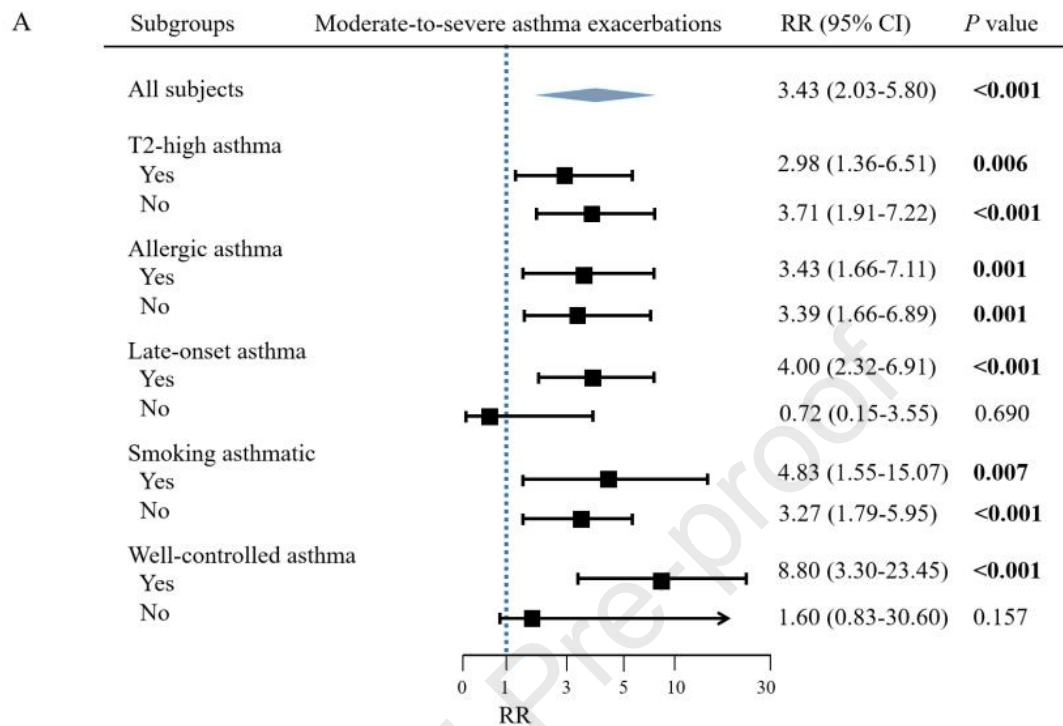
Figure 1. Flowchart of study. CV, coefficient variation; Ig, immunoglobulin; lg, logarithm base 10.

Figure 2. Comparison of airway inflammatory cytokines, including IFN- γ (A), TNF- α (B), IL-1 β (C), IL-4 (D), IL-5 (E), IL-6 (F), IL-8 (G) and IL-13 (H), between the IgE CV-high and IgE CV-low cohorts by Mann-Whitney U test. CV, coefficient variation; Ig, immunoglobulin; IFN, interferon; TNF, tumor necrosis factor; IL, interleukin. Bold values denote statistical significance at the $P < 0.05$.

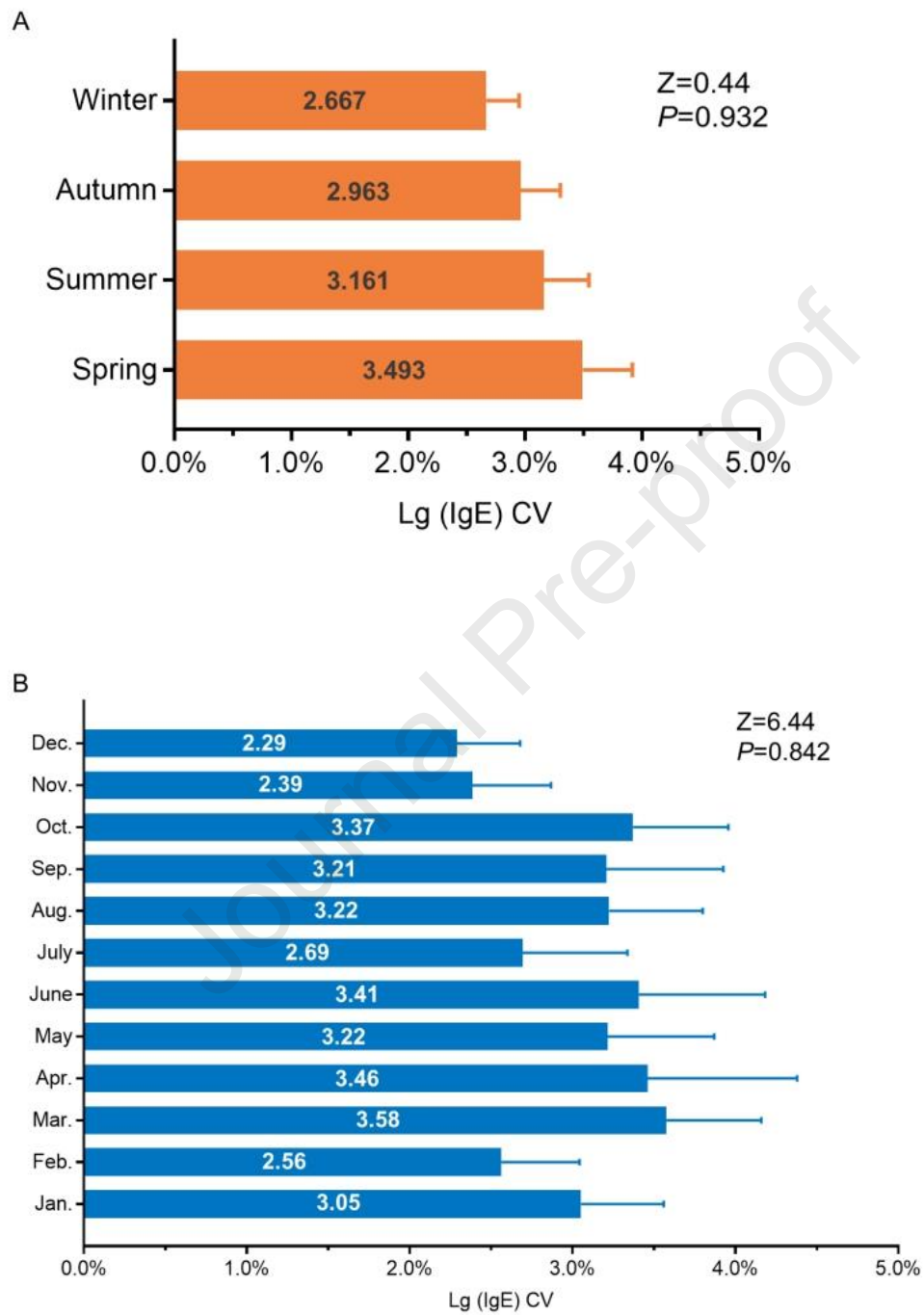
Figure 3. Adjusted rate ratios (RR_{adj}) for moderate to severe asthma exacerbations (A) and severe asthma exacerbations (B) in patients with IgE CV-high (vs. IgE CV-low) for one year follow-up by negative binominal regression models. *Adjusted for age, sex, BMI, smoking status, atopic status, FEV₁% predicted, ICS dosage, and baseline IgE level. BMI, body-mass index; CV, coefficient variation; FEV₁, forced expiratory volume in 1 second as; ICS, inhaled corticosteroid; Ig, immunoglobulin; lg, logarithm base 10; RR_{adj} , adjusted rate ratio; T2, type 2. Bold values denote statistical significance at the $P < 0.05$.

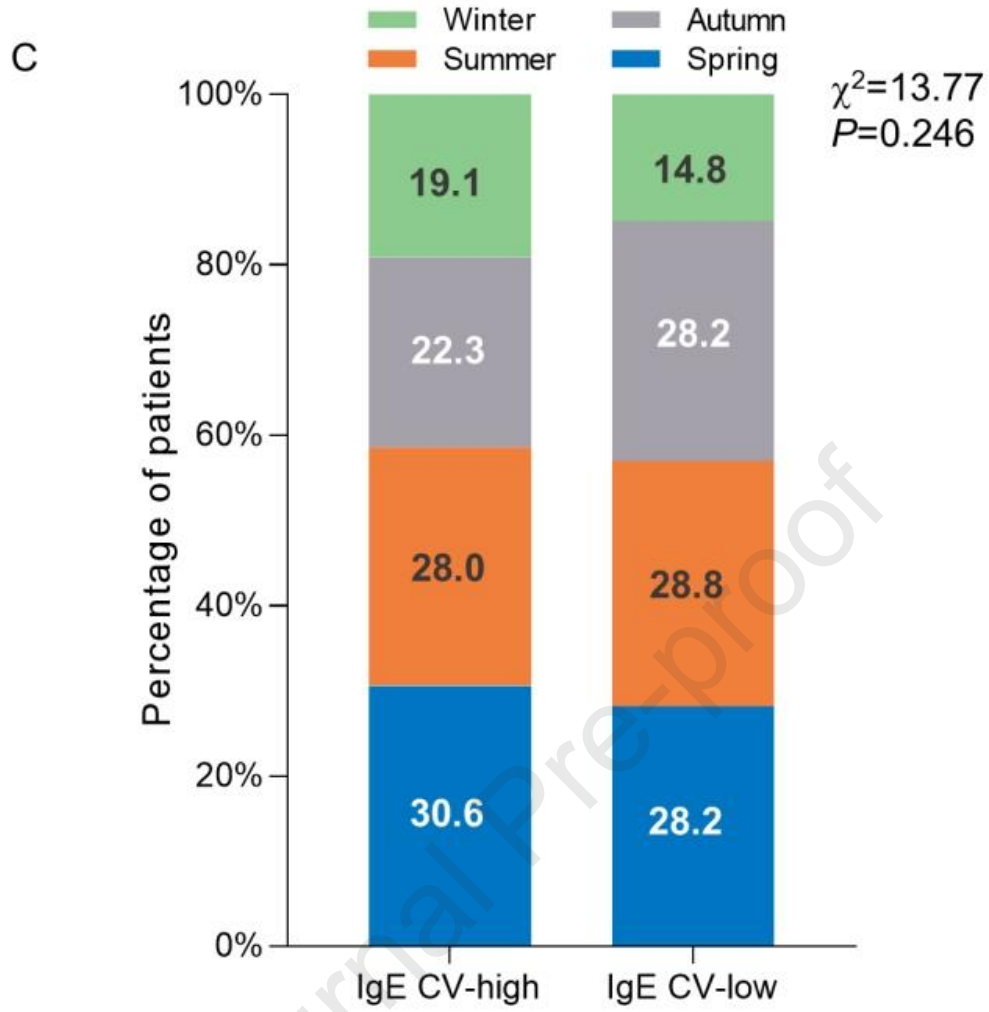
Figure 4. Multiple parallel mediation model of association of IgE CV with asthma exacerbations (AEs) using sputum cytokines as mediators after controlling for sex, age, BMI, smoking status, atopic status, FEV₁ % predicted, ICS dosage and baseline total IgE level. (A) Conceptual parallel mediation model; The mediation model of the association between IgE CV-high and moderate-to-severe AEs (B) or severe AEs (C) with sputum IL-6, IL-8 as mediator. a=effect of X on M; b=effect of M on Y; c'=direct effect of X on Y controlling for M; ab=indirect effect of X on Y through M (the product of path coefficients a and b); c=total effect of X on Y (direct and indirect). Each path presented as unstandardized coefficients (β) with their standard errors (SE). AE, asthma exacerbation; BC, bias-corrected; BMI, body mass index; CI, confidence interval; CV, coefficient variation; ICS, inhaled corticosteroid; Ig, immunoglobulin; IL, interleukin; IE, indirect effect. * $P < 0.05$

Repository E Figure No.1



Repository E Figure No.2





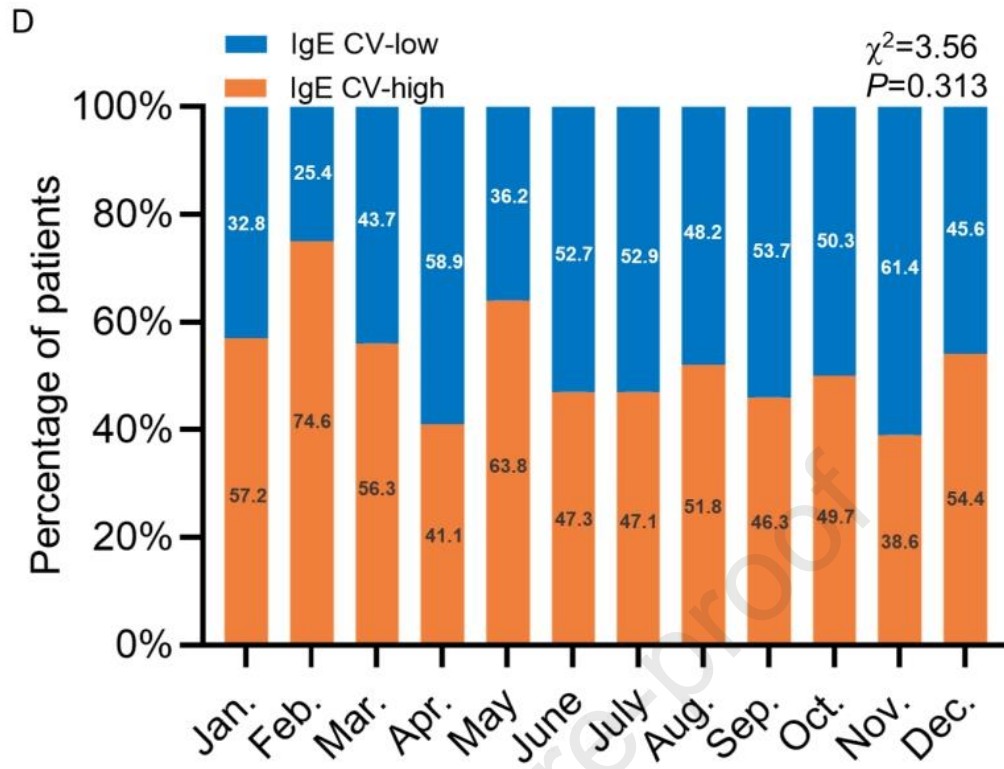


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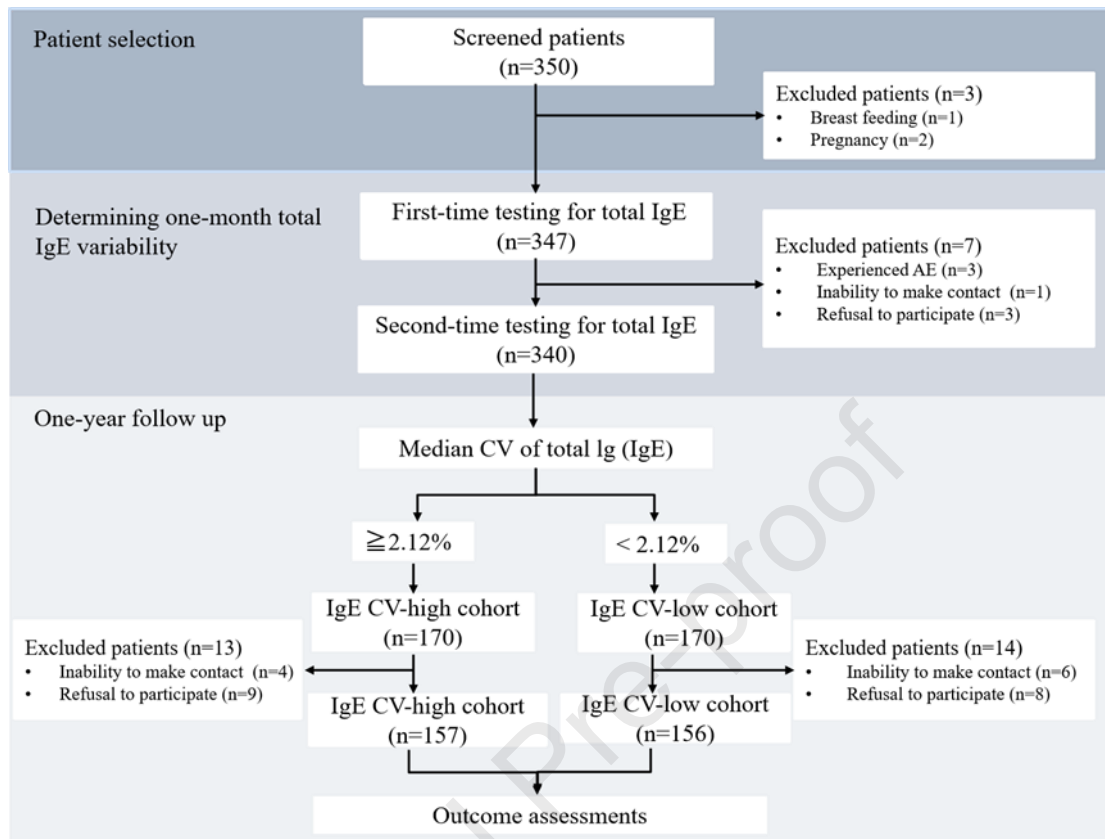


Figure No.2

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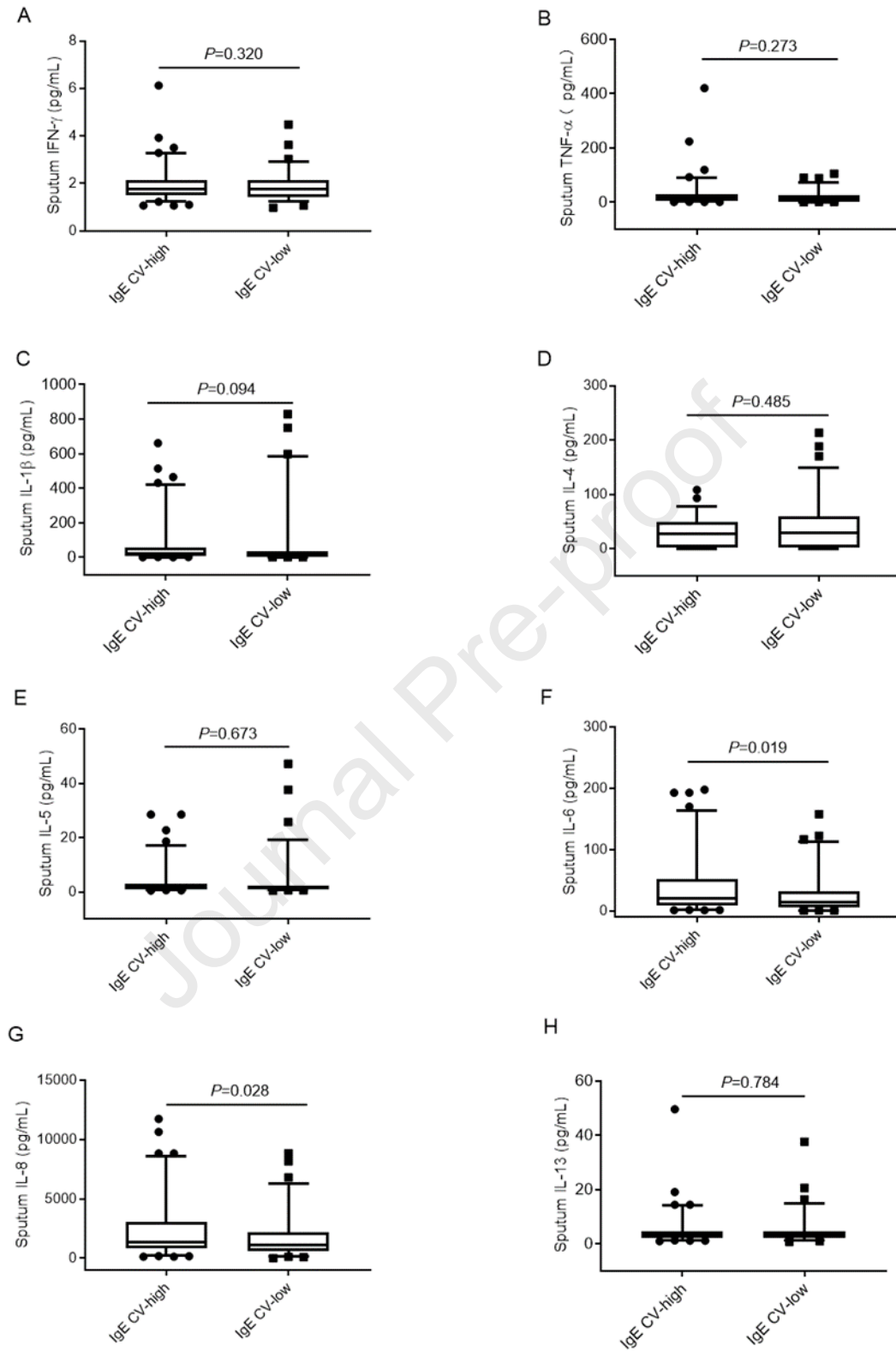


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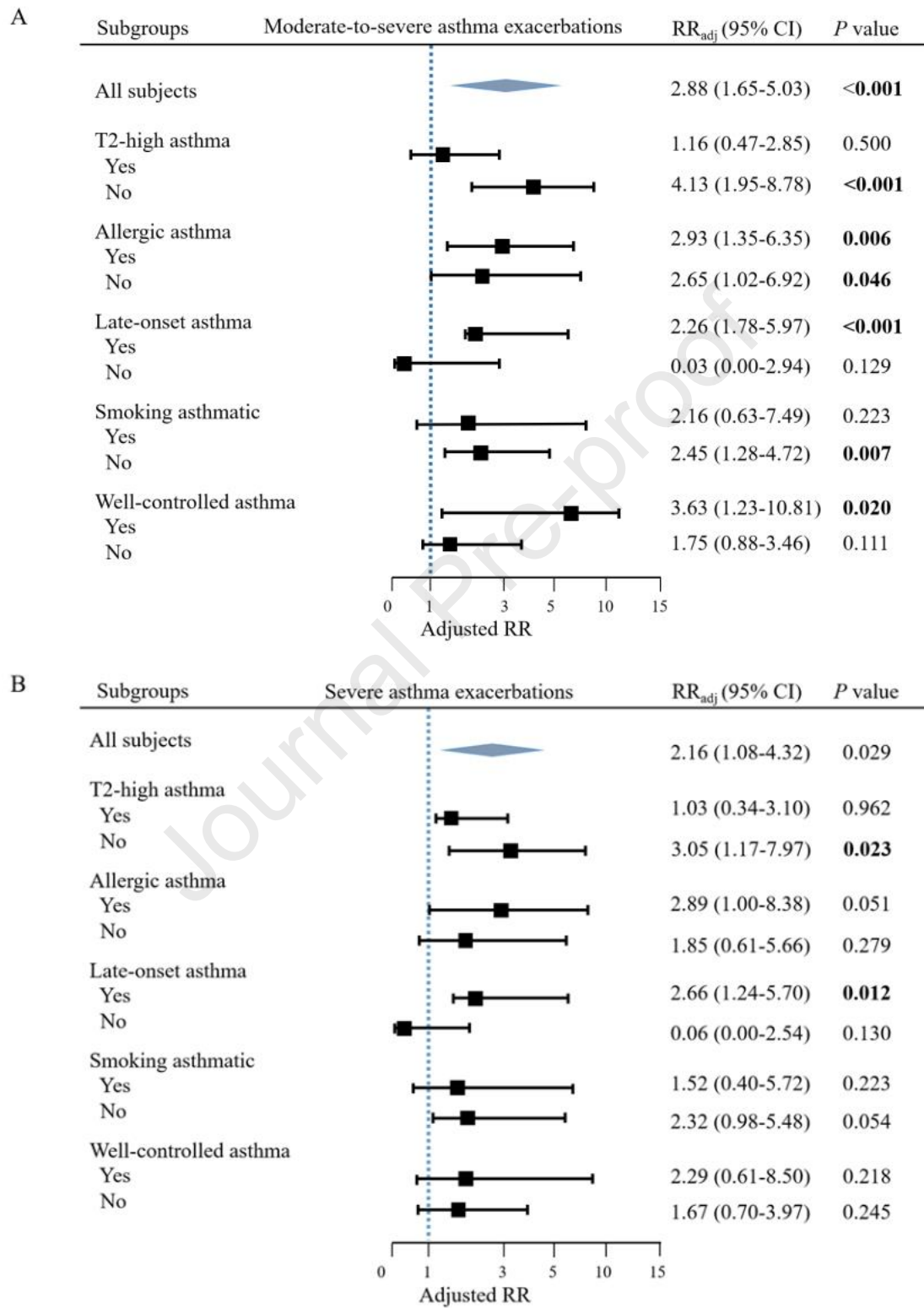
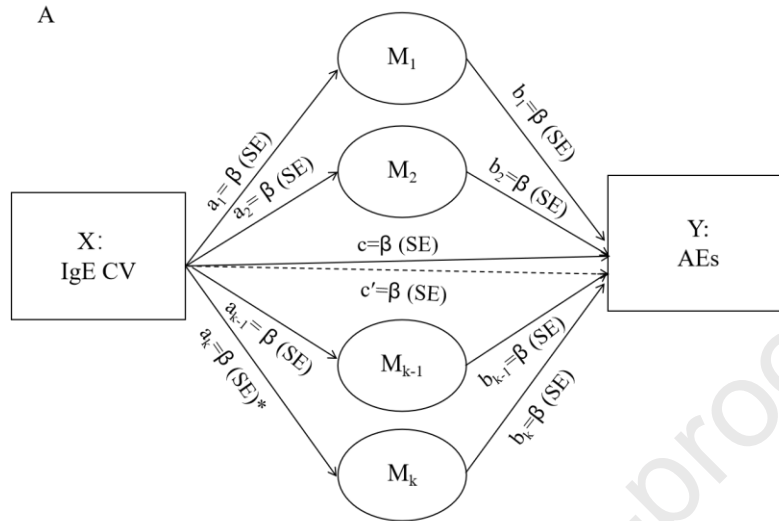
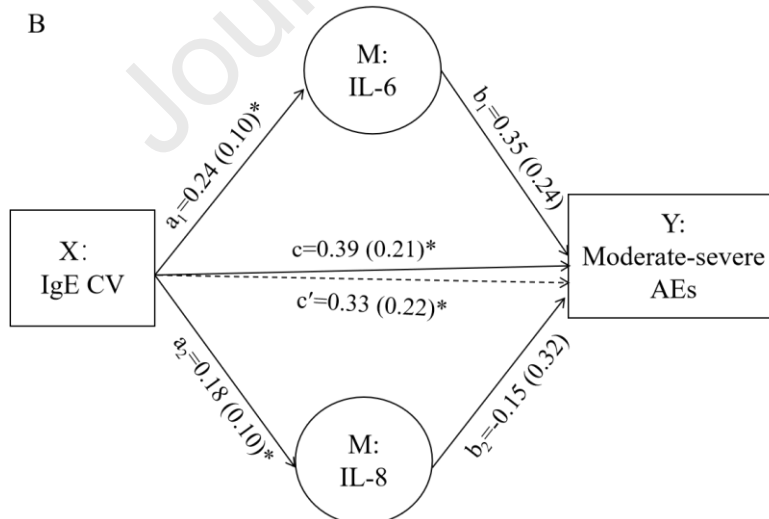


Figure No.4

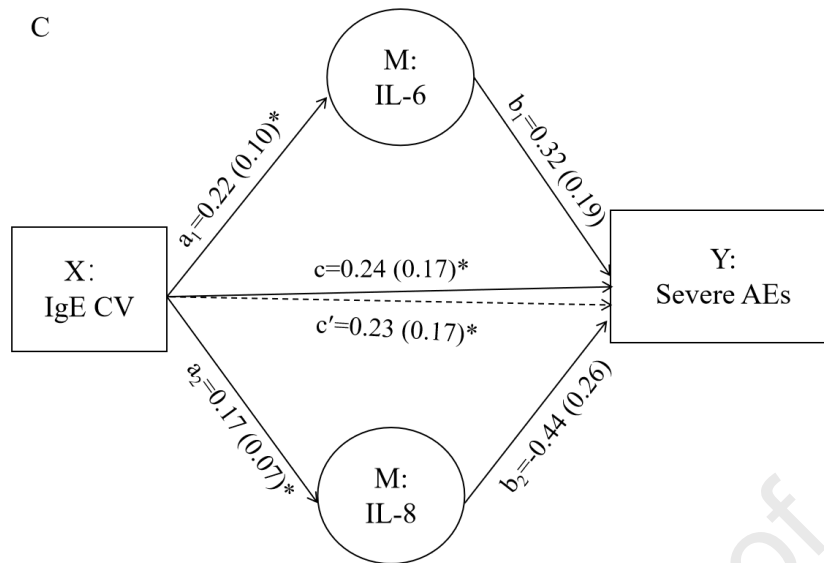


IE ($X \rightarrow M \rightarrow Y$) = point estimate (BC 95% CI) of $a*b$.



IE (IgE CV $\rightarrow$ sputum IL-6 $\rightarrow$ moderate-severe AEs) = 0.085 (0.002 to 0.200);
 IE (IgE CV $\rightarrow$ sputum IL-8 $\rightarrow$ moderate-severe AEs) = -0.020 (-0.116 to 0.058).

C



IE (IgE CV $\rightarrow$ sputum IL-6 $\rightarrow$ Severe AEs) = 0.070 (0.003 to 0.165);
 IE (IgE CV $\rightarrow$ sputum IL-8 $\rightarrow$ Severe AEs) = -0.063 (-0.184 to 0.005).

1 ONLINE REPOSITORY

2 **Total IgE variability is associated with future asthma exacerbations: a one-year** 3 **prospective cohort study**

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METHODS

Statistical analyses

To test the predictive value of a single blood sample of IgE (baseline lg [IgE]) combined with clinical history and complete blood cells (CBC) to AEs, we used multivariable negative binomial models (stepwise forward process), including the variables (with $P < 0.20$, in the univariate analysis) into the models^{E1}. Based on previous findings, clinical history-related factors, including sex, age, BMI, smoking, AEs in the previous year, asthma control level (ACT score), and comorbidities (such as sinusitis), are associated with future AEs^{E2}. Moreover, F_ENO, blood eosinophils, and neutrophils are also associated with AEs^{E3, 4}. Therefore, we included these variables in our negative binomial regression model.

In negative binomial regression, the level of the mean lg (IgE) (between baseline time and one month later) was treated as an independent variable, and AEs during the follow-up year were treated as dependent variables. The median of the mean lg (IgE) level was 2.133 IU/mL. The mean lg (IgE) was coded as a binary variable based on its

median (≥ 2.133 IU/mL and < 2.133 IU/mL were coded as “1” and “0,” respectively).

The adjusted confounders in this analysis included sex, age, BMI, smoking status, atopic status, FEV₁% predictive, ICS dosage, baseline IgE level, and AEs in the previous year.

Mediation analyses

Generally, a mediation model seeks to identify and explain the mechanism or process that underlies an observational relationship between an independent variable and a dependent variable via the inclusion of a third hypothetical variable, known as a mediator variable^{E5}. A mediation model proposes that the independent variable influences the (non-observable) mediator variable, which in turn influences the dependent variable, instead of a direct causal relationship between the independent and dependent variables^{E5}. Thus, the mediator variable serves to clarify the nature of the relationship between the independent and dependent variables.

In our study, both sputum IL-6 and IL-8 levels were associated with IgE CV (Table 3). The parallel multiple indirect effect model allowed for potential mediator variables to be correlated, but not exert a causal impact among the variables^{E6}. Therefore, we used a parallel multiple mediation model to examine whether the relationship between IgE CV (a dichotomous independent variable, IgE CV-high vs. IgE CV-low, X) and AEs (dependent variable, Y) was mediated by sputum IL-6 and/or IL-8 (as mediator variables, M) (Figure 4 A). Confounders, such as sex, age, BMI, smoking status, atopic status, FEV₁ % predictive value, ICS dosage, and baseline total IgE level, were adjusted in the model.

Mediation hypotheses were tested via bootstrapping with 10,000 samples to calculate bias-corrected confidence intervals (BC 95% CI) with the PROCESS macro (version 3.3, model 4) for SPSS 22 using ordinary least squares regression to estimate variables on the left side of the model equations^{E5}. Significance was achieved when an indirect effect ($a \times b$) was observed and zero was not included in the confidence

intervals, instead of individual tests of the paths of the model (c, a, and b)^{E5}. Specifically, when computing the correlation coefficients between X (IgE CV-high) and M (sputum IL-6/IL-8) (path a), between M (sputum IL-6/IL-8) and Y (AEs) (path b), and between X (IgE CV-high) and Y (AEs) (path c, the total effect [which includes the influence of M]), the direct effect of X on Y (path c' [c prime]) controls for the influence of the mediator. Thus, the indirect effect (ab: the product of path coefficients a and b) quantifies the strength or influence of the mediator on the X–Y relationship.

Mediation hypotheses were tested using bias-corrected bootstrapping with 10,000 samples to calculate confidence intervals (95%) using the PROCESS macro (version 3.3) for SPSS 22 using ordinary least squares (OLS) regression to estimate variables on the left sides of the model equations.

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112 **E-Tables**

113

Table E1. Associations of sputum cytokines with IgE CV-high cohort by logistic regression model

Cytokines in sputum, pg/mL [#]	OR (95% CI)	P-value
IFN- γ	0.248 (0.028–2.187)	0.209
TNF- α	1.39 (0.78–2.48)	0.271
IL-1 β	1.51 (0.93–2.45)	0.096
IL-4	0.93 (0.65–1.34)	0.698
IL-5	0.96 (0.46–2.01)	0.906
IL-6	1.93 (1.10–3.36)	0.021
IL-8	2.27(1.08–4.77)	0.030
IL-13	1.11 (0.41–3.01)	0.846
BMI, body mass index; CI: confidence interval; CV: coefficient of variation; IFN, interferon; IL: interleukin; Ig, immunoglobulin; OR: odds ratio; TNF: tumor necrosis factor. [#] Sputum cytokine values have been base-10 log-transformed. Bold values denote statistical significance at the $P < 0.05$.		

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Table E2. The predictive properties of high IgE CV for moderate-to-severe AE in the following year

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Total sample	66.7	53.1	21.7	89.1
Subgroups				
T2-high/non-T2 high	56.3/70.6	50.5/54.5	14.3/25.5	88.7/89.4
Allergic/non-allergic	72.7/62.1	53.4/53.6	17.6/28.6	93.5/82.5
Late-onset/early-onset	67.4/40.0	53.7/47.9	23.8/7.4	88.5/88.5
Smoking/non-smoking	75.0/62.9	57.6/51.5	30.0/18.8	90.5/88.6
Well-controlled/partial and uncontrolled	79.0/54.8	54.3/51.5	20.5/20.5	94.5/83.3
AE, asthma exacerbation; CV, coefficient of variation; Ig, immunoglobulin; lg, logarithm base 10; NPV, negative predictive value; PPV, positive predictive value; T2, type 2.				

Table E3. The predictive properties of high IgE CV for severe AE in the following year

	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Total	64.9	51.8	15.3	91.7
Subgroups				
T2-high/non-T2 high	50.0/73.9	59.5/53.3	11.1/18.1	88.7/93.6
Allergic/non-allergic	73.3/54.5	52.4/51.9	12.1/18.8	95.7/84.8
Late-onset/early-onset	68.8/40.0	52.6/50.0	16.9/7.7	92.3/88.9
Smoking/non-smoking	66.7/64.0	54.3/51.0	20.0/13.7%	90.5/92.1
Well-controlled/partial and uncontrolled	76.9/58.3	52.6/51.7	13.7/16.9	95.9/88.1
AE, asthma exacerbation; CV, coefficient of variation; Ig, immunoglobulin; lg, logarithm base 10; NPV, negative predictive value; PPV, positive predictive value; T2, type 2.				

Table E4. The associations of baseline IgE level/Ig (IgE) CV (combined with clinical history and CBC) and moderate-to-severe AEs in the following year						
Variables	Univariate analysis		Multi-variate negative binomial final model			
			Baseline Ig (IgE)		Ig (IgE) CV-high	
	RR (95% CI)	P-value	RR (95% CI)	P-value	RR (95% CI)	P-value
IgE						
Baseline Ig (IgE), IU/mL	0.78 (0.54–1.13)	0.189#	0.92 (0.57–1.49)	0.746		
Ig (IgE) CV-high§	3.43 (2.03–5.80)	<0.001#			2.10 (1.18–3.74)	0.011*
Clinical history and CBC						
Female	0.72 (0.46–1.15)	0.167#	0.78 (0.45–1.35)	0.377	0.78 (0.45–1.36)	0.385
Age, years	1.02 (1.00–1.03)	0.053#	1.02 (1.00–1.04)	0.136	1.01 (0.99–1.04)	0.158
BMI, kg/m ²	1.03 (0.97–1.10)	0.319				

Bold values denote statistical significance at the $P<0.05$.

Table E5. The associations of baseline IgE level/ lg (IgE) CV (combined with clinical history and CBC) and severe AEs in the following year						
Variables	Univariate analysis		Multi-variate negative binomial final model			
			Baseline lg (IgE)		lg (IgE) CV-high	
	RR (95% CI)	P-value	RR (95% CI)	P-value	RR (95% CI)	P-value
IgE						
Baseline lg (IgE), IU/mL	0.75 (0.48–1.16)	0.199#	0.72 (0.40–1.28)	0.716		
lg (IgE) CV-high§	3.25 (1.75–6.03)	< 0.001 #			2.07 (1.06–4.05)	0.034
Clinical history and CBC						
Female	0.76 (0.47–1.21)	0.242				
Age, years	1.01 (0.99–1.03)	0.310				
BMI, kg/m ²	0.99 (0.91–1.07)	0.813				
Smoking	1.98 (1.12–3.51)	0.019 #	0.92 (0.44–1.93)	0.834	1.06 (0.52–2.18)	0.877

ACT score	0.95 (0.90–1.01)	0.127 #	0.95 (0.90–1.01)	0.120	1.01 (0.94–1.09)	0.792
AEs in the previous year	1.25 (1.10–1.42)	0.001#	1.20 (1.05–1.38)	0.010	1.21 (1.05–1.40)	0.009
Sinusitis	1.62 (0.93–2.83)	0.090 #	1.45 (0.73–2.90)	0.294	1.21 (0.61–2.41)	0.582
F _E NO, ppb	1.001 (0.993–1.007)	0.983				
Neutrophils, ×10 ⁹ /L	1.04 (0.86–1.25)	0.694				
Neutrophils, %	1.00 (0.97–1.03)	0.856				
Eosinophils, ×10 ⁹ /L	1.70 (0.67–4.29)	0.265				
Eosinophils, %	1.00 (0.93–1.07)	0.923				

ACT, asthma control test; AE, asthma exacerbation; BMI, body mass index; CBC, complete blood cells; CI, confidence interval; F_ENO: fractional exhaled nitric oxide; HAD-A, hospital anxiety and depression scale-anxiety; HAD-D, hospital anxiety and depression scale-depression; Ig, immunoglobulin; lg, logarithm base 10

§ Compared with a reference group of patients with lg (IgE) CV-low; # Variables with *P*<0.20 in the univariate analysis were included into the multi-variate negative binomial final model.

Bold values denote statistical significance at the *P*<0.05.

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Table E6. Associations of lg (IgE) mean or lg (IgE) CV with moderate-to-severe asthma exacerbations in the following year

	lg (IgE) mean		lg (IgE) CV	
Participants	RR _{adj} (95% CI) *	P-value	RR _{adj} (95% CI) *	P-value
Total (n=313)	0.56 (0.28–1.12)	0.102	2.88 (1.65–5.03)	<0.001
Subgroups				
T2-high				
Yes	0.11 (0.02–0.60)	0.011	1.16 (0.47–2.85)	0.500
No	2.83 (1.38–5.80)	0.005	4.13 (1.95–8.78)	<0.001
Allergic				
Yes	0.24 (0.08–0.74)	0.013	2.93 (1.35–6.35)	0.006
No	2.12 (0.82–5.50)	0.121	2.65 (1.02–6.92)	0.046
Late-onset				
Yes	0.48 (0.23–0.99)	0.047	2.26 (1.78–5.97)	<0.001
No	0.39 (0.07–2.16)	0.279	0.03 (0.00–2.94)	0.129
Smoking				
Yes	1.06 (0.22–5.19)	0.943	2.16 (0.63–7.49)	0.223
No	0.28 (0.12–0.66)	0.003	2.45 (1.28–4.72)	0.007
Well-controlled#				
Yes	0.25 (0.06–1.03)	0.540	3.63 (1.23–10.81)	0.020

No	0.81 (0.35–1.85)	0.611	1.75 (0.88–3.46)	0.111
*Negative binominal regression models were used to explore the associations of lg (IgE) mean with moderate-to-severe AEs by adjusting for sex, age, BMI, smoking status, atopic status, FEV₁ % predictive, ICS dosage, baseline IgE level, and AEs in the previous year. # Asthma control was determined by ACT score, scores of 20–25 classified as well-controlled asthma; 16–19 as partially controlled, and 5–15 as uncontrolled asthma. ACT, asthma control test; AE, asthma exacerbation; BMI, body mass index; CI, confidence interval; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; GINA, Global Initiative for Asthma; ICS, inhaled corticosteroid; Ig, immunoglobulin; lg, logarithm base 10; RR_{adj}, adjusted rate ratio; T2, type 2. Bold values denote statistical significance at the $P<0.05$.				

Table E7. Associations of lg (IgE) mean or lg (IgE) CV with severe asthma exacerbations in the following year

	lg (IgE) mean		lg (IgE) CV	
Participants	RR _{adj} (95% CI) *	P-value	RR _{adj} (95% CI) *	P-value
Total (n=313)	0.69 (0.29–1.64)	0.693	2.16 (1.08–4.32)	0.029
Subgroups				
T2-high				
Yes	0.18 (0.02–1.15)	0.066	1.03 (0.34–3.10)	0.962
No	1.91 (0.72–5.05)	0.195	3.05 (1.17–7.97)	0.023
Allergic				
Yes	0.12 (0.02–0.66)	0.015	2.89 (1.00–8.38)	0.051
No	1.03 (0.30–3.49)	0.964	1.85 (0.61–5.66)	0.279
Late-onset				
Yes	0.66 (0.26–1.62)	0.361	2.66 (1.24–5.70)	0.012
No	18.46 (0.72–47.32)	0.303	0.06 (0.00–2.54)	0.130
Smoking				
Yes	0.44 (0.06–3.32)	0.428	1.52 (0.40–5.72)	0.223
No	0.39 (0.13–1.10)	0.076	2.32 (0.98–5.48)	0.054
Well-controlled #				
Yes	0.79 (0.12–5.04)	0.803	2.29 (0.61–8.50)	0.218
No	0.93 (0.33–2.63)	0.886	1.67 (0.70–3.97)	0.245
*Negative binominal regression models were used to explore the relationship of lg (IgE) mean with severe AEs by adjusting for sex, age, BMI, smoking status, atopic status, FEV₁ % predictive, ICS dosage, baseline total IgE level, and AEs in the previous year. # Asthma control was determined by ACT score, scores of 20–25 classified as well-controlled asthma, 16–19 as partial-controlled, and 5–15 as uncontrolled asthma. RR_{adj}, adjusted rate ratio. ACT, asthma control test; AE, asthma exacerbations; BMI, body mass index; CI, confidence interval.				

FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; GINA, Global Initiative for Asthma; ICS, inhaled corticosteroid; Ig, immunoglobulin; lg, logarithm base 10; T2, type 2.

Bold values denote statistical significance at the $P<0.05$.

Table E8. Demographic and clinical characteristics of the patients in one-month cohort and the validation three-month cohort

Characteristics	One-month cohort	Three-month cohort	$t/Z/\chi^2$	P-value
n	340	114		
Age, years, median (Q1, Q3)	43.81 (35.00, 53.93)	46.50 (35.00, 55.00)	-1.332	0.183 ^{††}
Female, n (%)	200 (63.9)	67 (64.4)	0.009	0.923 ^{†††}
BMI, kg/m ² , median (Q1, Q3)	23.12 (20.96, 25.15)	22.08 (20.45, 24.56)	-1.539	0.124 ^{††}
Smoking, pack-years, mean (SD)	3.11 (8.96)	2.30 (7.42)	-1.476	0.140 ^{††}
Atopic status, n (%)	197 (57.9)	65 (57.0)	0.012	0.912 ^{†††}
Family history, n (%)	117 (34.4)	38 (33.3)	0.246	0.620 ^{†††}
Age of onset, years, median (Q1, Q3)	33.00 (21.25, 44.00)	33.00 (19.00, 48.00)	-0.453	0.651 ^{†††}
ACT score, median (Q1, Q3)	19 (16, 22)	21 (15, 23)	-1.387	0.165 ^{†††}
AQLQ score, median (Q1, Q3)	5.83 (5.18, 6.36)	5.86 (5.25, 6.31)	-0.137	0.891 ^{††}
Medications				
ICS dosage, BDP equivalent, µg/d, mean (SD)	582.64 (500.89)	565.79 (391.01)	-1.185	0.236 ^{††}
ICS/LABA, n (%)	195 (57.4)	62 (54.4)	0.306	0.580 ^{†††}
OCS, n (%)	12 (3.8)	5 (4.4)	0.174	0.677 ^{†††}

GINA steps 4–5, n (%)	80 (23.5)	29 (25.4)	0.171	0.680†††
Spirometry				
FEV ₁ , L, mean (SD)	2.08 (0.77)	2.04 (0.75)	−1.304	0.192†
FEV ₁ , % predicted, median (Q1, Q3)	72.5 (57.0, 88.0)	72.0 (59.0, 87.0)	−0.421	0.674††
FVC, L, median (Q1, Q3)	3.09 (2.56, 3.61)	2.91 (2.57, 3.62)	−1.268	0.205††
FEV ₁ /FVC, %, mean (SD)	66.23 (13.89)	65.37 (13.11)	−0.317	0.751†
Asthma phenotypes				
Inflammatory phenotypes [#] , EA/NA/PGA, n (%)	114 (43.5)/43 (16.4)/105 (40.1)	38 (38.4)/20 (20.2)/41 (41.4)	3.565	0.312†††
Late-onset asthma, n (%)	284 (83.5)	94 (82.5)	0.071	0.791†††
T2-high asthma, n (%)	161 (47.5)	47 (42.7)	0.758	0.384†††
Allergic asthma, n (%)	197 (57.9)	65 (57.1)	0.012	0.912†††
Serum total IgE at baseline, IU/mL, median (Q1, Q3)	142.64 (43.41, 329.54)	147 (56.83, 288.50)	−0.455	0.649††
Serum total IgE one-month later, IU/mL, median (Q1, Q3)	125.22 (45.59, 323.66)	132.5 (55.68, 288.50)	−0.801	0.423††

Lg (IgE) CV, %, median (Q1, Q3)	2.12 (0.98, 3.91)	2.32 (1.33, 3.78)	-1.016	0.310††
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ACT, Asthma Control Test; AQLQ, Asthma Quality of Life Questionnaire; BDP, beclomethasone equivalents; BMI, body mass index; CV, coefficient of variation; EA, eosinophilic asthma; FEV₁, forced expiratory volume in 1 second; FVC, forced vital capacity; GINA, Global Initiative for Asthma; ICS, inhaled corticosteroid; ICS/LABA, inhaled corticosteroid with long-acting beta-agonist; Ig, immunoglobulin; lg, logarithm base 10; NA, neutrophilic asthma; OCS, oral corticosteroid; Q1, first quartile; Q3, third quartile; PGA: paucigranulocytic asthma; SD, standard deviation; T2, type 2.

Mixed granulocytic asthma was not analyzed since only one patient was identified as granulocytic asthma; eosinophilic asthma was defined as sputum eosinophils $\geq 3\%$ alone and/or blood eosinophils $\geq 0.3 \times 10^9$ cells/L; neutrophilic asthma was defined as sputum neutrophil count $\geq 61\%$ and eosinophil count $< 3\%$; paucigranulocytic asthma was defined as sputum neutrophil count $< 61\%$ and eosinophil count $< 3\%$.

Statistical analyses were performed by † Student's t test, †† Mann-Whitney U test, ††† Chi-square test.

Table E9. Intraclass correlation coefficients of lg IgE CV of the patients in two cohorts

Cohorts	IgE, IU/mL, median (Q1, Q3)			ICCs of lg (IgE)	P-value	Reproducibility*
	Baseline	One month	Three months			
One-month cohort (n=340)	142.64 (43.41, 329.54)	147 (56.83, 288.50)	NA	0.977	< 0.001 **	Excellent
Three-month cohort (n=114)	125.22 (45.59, 323.66)	132.50 (55.68, 288.50)	123.12 (46.38, 286.76)	0.979	< 0.001 **	Excellent

Ig, immunoglobulin; lg, logarithm base 10; ICC, intraclass correlation coefficient; Q1, first quartile; Q3, third quartile.

Two-way random-effects model.

* ICC was ranged from 0 (low reproducibility) to 1 (high reproducibility). Standardized criteria proposed by Rosner (2000) to evaluate ICCs: ICC <0.40 was assessed as poor reproducibility; $0.40 \leq \text{ICC} < 0.75$ as fair to good reproducibility, and $\text{ICC} \geq 0.75$ as excellent reproducibility.

** There was no significant difference of the ICCs between the two cohorts ($Z=0.4$, $P=0.689$).

Bold values denote statistical significance at the $P<0.05$.

E-Figure Legends

Figure E1. Rate ratios (RRs) for moderate to severe asthma exacerbations (A) and severe asthma exacerbations (B) in patients with IgE CV-high (vs. IgE CV-low) during one-year follow-up by negative binominal regression models without adjusting confounders. CV, coefficient variation; Ig, immunoglobulin; lg, logarithm base 10; RR, rate ratio; T2, type 2. Bold values denote statistical significance at $P<0.05$.

Figure E2. The distributions of sampling time. The seasonal (A) and monthly (B) distributions of lg (IgE) CV (mean $\pm$ SE) tested by Mann-Whitney U test; the patient proportion in different seasons (C) and months (D) between the lg (IgE) CV-high and lg (IgE) CV-low cohorts tested by Chi-square test.