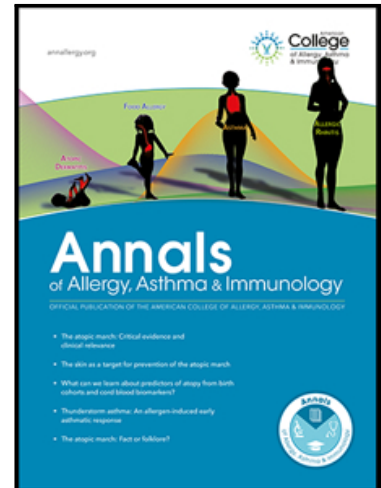


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Serum Free IgE: a Useful Biomarker of Atopy and Type 2 Asthma in Adult Asthmatics

Seong-Dae Woo, MD^a, Eun-Mi Yang^a, Jaehyuk Jang, MD^a, Youngsoo Lee, MD^a, Yoo Seob Shin, MD, PhD^a, Young-Min Ye, MD, PhD^a, Su Youn Nam, MD, PhD^b, Kyung Wha Lee, PhD^b, Myoung Ho Jang, PhD^b, Hae-Sim Park, MD, PhD^a Suwon and Seoul, South Korea

^aDepartment of Allergy and Clinical Immunology, Ajou University School of Medicine, Suwon, Korea

^bGI Innovation, Seoul, South Korea

Corresponding author: Hae-Sim Park, MD, PhD, Department of Allergy and Clinical Immunology, Ajou University School of Medicine, 164 World cup-ro, Yeongtong-gu, Suwon 16499, Korea. E-mail: hspark@ajou.ac.kr

Tel: +82-31-219-5000

Fax: +82-31-219-6380

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Author contributions

All authors have made substantial contributions to the conception and design of the study. SDW, EMY, JJ and YL performed the experiments and related data analysis. SDW, JJ, YL, YSS and YMY collected the clinical data. SYN, KWL and MHJ developed the IgE_{TRAP} protein. SDW wrote the manuscript. HSP supervised all the process. All authors reviewed and approved the final manuscript.

Abstract

BACKGROUND: It has been known that a high serum total IgE level is a predisposing factor of allergic asthma; however, there are considerable limitations to apply it in clinical practice.

OBJECTIVE: The aim of this study is to determine the clinical significance of the serum free IgE level in patients with adult asthma.

METHODS: We measured free IgE levels using our home-made enzyme-linked immunosorbent assay (ELISA) via applying a novel IgE TRAP protein (GI innovation, Seoul, Korea) in sera of adult asthmatics (n = 116) compared to healthy controls (n = 32); ELISA inhibition test was performed to validate its binding specificity. Associations between asthma-related clinical and laboratory parameters were analyzed. The diagnostic value and cutoff point for detecting atopy and type 2 asthma were determined using receiver operating characteristic (ROC) curve analysis.

RESULTS: The serum free IgE levels were significantly higher in adult asthmatics than in healthy controls and were significantly associated with atopic status and type 2 asthma (all $P < .001$). In the ROC analysis, serum free IgE had a significantly greater area under the curve (AUC) than serum total IgE for assessing asthma, especially type 2 asthma (AUC = 0.810 vs 0.743, $P = .006$ and AUC = 0.729 vs 0.572, $P < .001$). The optimal cutoff points for predicting atopy and type 2 asthma were 82.8 and 120.8 ng/mL, respectively.

CONCLUSION: It is suggested that a higher serum free IgE level may be a useful biomarker of atopy and type 2 asthma in adult asthmatics.

Key words: *Airway remodeling; Monoclonal antibodies; Asthma; Biomarkers; Enzyme-linked immunosorbent assay; Eosinophils; Immunoglobulin E*

Abbreviations used

AUC- Area under the curve

ELISA- Enzyme-linked immunosorbent assay

FeNO- Fraction of exhaled nitric oxide

FEV₁- Forced expiratory volume in 1 second

FVC- Forced vital capacity

GINA- Global Initiative for Asthma

PBS- Phosphate-buffered saline

ROC- Receiver operating characteristic

INTRODUCTION

IgE is well known as a key mediator in the development and maintenance of allergic inflammation. It is known to have 2 principal receptors: the high-affinity receptor for IgE (FcεRI) is expressed on tissue mast cells, blood basophils, airway smooth muscle cells, and antigen-presenting cells.^{1, 2} Cross-linking of IgE-FcεRI complexes cause the release of inflammatory mediators and cytokines from IgE effector cells, which contributes to eosinophil recruitment into inflammatory tissue.^{3, 4} FcεRII (CD23), the low-affinity receptor for IgE, plays a central role in enhancing the activation and proliferation of allergen-specific T cells through the process called IgE-facilitated allergen presentation.^{5, 6} T cell activation by CD23 increases the levels of type 2 cytokines, such as IL-4, IL-5, and IL-13, which are responsible for the late phase of allergic inflammations.⁵

Despite the importance of IgE in allergic airway disease, currently available methods for the measurement of serum total IgE have limitations to apply in clinical practice due to a wide overlap in the distribution of its concentrations between atopic and nonatopic individuals.^{7, 8} Elevated levels of serum total IgE are found in patients not only with allergic asthma, but also with other conditions such as parasitic infestations, inflammatory diseases, hematologic malignancies, and some primary immunodeficiency diseases, while low or normal values do not exclude the presence of allergic asthma.^{8, 9} Consequently, total IgE levels could not be considered an independent marker for the diagnosis of allergic asthma.¹⁰ Furthermore, serum total IgE levels include both complex form of IgE and free IgE.¹ In particular, total IgE levels are increased after omalizumab treatment in severe asthmatics due to the detection of IgE-omalizumab complexes, which makes it impossible to monitor therapeutic responses and determine the appropriate dose of omalizumab.^{11, 12} Alternatively,

serum free IgE levels are decreased up to 95% after omalizumab treatment in patients with clinical responses, suggesting that it could be used to determine therapeutic responses.¹¹⁻¹⁴

To date, there has been no reliable method for the measurement of serum free IgE. Its clinical and diagnostic value has not been investigated. The present study aimed (1) to establish a new technique for detecting serum free IgE using a novel IgE_{TRAP} protein and (2) to analyze the associations between clinical and laboratory parameters in order to provide relevant cutoff points for representing atopy or the endotype of asthma.

METHODS

Study subjects

One hundred sixteen adult patients with a diagnosis of asthma based on clinical symptoms (such as cough, dyspnea, chest tightness and wheezing), airway reversibility (an increase in FEV₁ $\geq$ 12% and 200 mL from pre-bronchodilator use), and airway hyper-responsiveness (provocative concentration of methacholine that results in a 20% decrease in FEV₁ $\leq$ 16mg/mL) were recruited from Ajou University Hospital in South Korea. All subjects had no history of any chronic or current acute disease. Patients with the use of biologic agents were excluded from the study. Their demographic characteristics were collected, including age, sex, and past history. A control group consisted of 32 healthy subjects with neither history of asthma nor other chronic diseases for comparative analyses. All subjects agreed to participate voluntarily in the study and provided written informed consent. This study was approved by the Institutional Review Board of Ajou University Hospital (AJIRB-BMR-SMP-19-001; AJIRB-BMR-SUR-15-498).

Data collection

All clinical data were obtained by clinical investigators and stored in a database. The presence of atopy was determined by a positive skin prick test (allergen/histamine ratio of wheal size ≥ 1) and/or elevated serum specific IgE level (≥ 0.35 IU/mL) to at least 1 common aeroallergen (pollens, house dust mites, animal dander, or mold). Spirometry with bronchodilator and methacholine challenge test were conducted, and peripheral eosinophil counts and fraction of exhaled nitric oxide (FeNO) levels (NIOX; Aerocrine AB, Solna, Sweden) were obtained. The serum levels of total IgE were measured with the ImmunoCAP system (ThermoFisher, Waltham, MA, USA). Serum periostin levels were measured by using commercial enzyme-linked immunosorbent assay (ELISA) kits (Shino-Test Corporation, Tokyo, Japan), according to the manufacturers' instructions. Induced sputum samples were collected by inhalation of nebulized hypertonic saline solution and dispersed using dithiothreitol for sputum cell count assessment. Sputum eosinophils were counted as a percentage of total sputum leukocytes.

Definition of type 2 asthma and asthma control status

Type 2 airway inflammation was defined on the basis of the Global Initiative for Asthma (GINA) guidelines.¹⁵ Patients with type 2-high asthma were defined according to either blood eosinophil count $\geq 150/\mu\text{L}$ and/or FeNO ≥ 20 ppb and/or sputum eosinophils $\geq 2\%$. On the contrary, patients with type 2-low asthma were defined according to blood eosinophil count $< 150/\mu\text{L}$ and FeNO < 20 ppb and sputum eosinophils $< 2\%$. Asthma control status was also assessed according to the GINA guidelines.¹⁵ Patients with well controlled status were classified as the controlled asthma group, whereas patients in partly controlled and uncontrolled status were classified as the uncontrolled asthma group. A cut-off point of 150

IU/mL was used to determine elevated serum total IgE.¹⁶

Preparation of IgE_{TRAP} protein

The IgE_{TRAP} protein, developed by GI innovation (Seoul, South Korea), comprises a human FcεRIα extracellular domain fused to a human IgG4 Fc domain (eFigure 1, A). The FcεRIα domain of IgE_{TRAP} has a high binding affinity for the Fc chain of IgE, which allows effective detection of circulating IgE.¹ This IgE_{TRAP}-Fc fusion protein was expressed in dihydrofolate reductase-deficient Chinese hamster ovary (CHO) cells transfected with α-2, 6-sialyltransferase gene. The IgE_{TRAP} protein was purified using an Amsphere A3 column (JSR, Ibaraki, Japan) and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing and non-reducing conditions and size exclusion high performance liquid chromatography (eFigure 1, B and C).

Measurement of free IgE in the sera of the study subjects

Measurement of serum free IgE was made using ELISA with applying a novel IgE_{TRAP} protein as previously described.¹⁷ Briefly, IgE_{TRAP} protein was used to coat ELISA plates with 0.1 μg/well and incubated overnight at 4°C. After washing 3 times with 0.05% phosphate-buffered saline (PBS)-Tween, the remaining binding sites were blocked with 300μL of blocking buffer (PBS containing 1% BSA) for 30 minutes. Diluted serum (1:10) from patients or control subjects was incubated for 1 hour at room temperature in IgE_{TRAP} protein-coated wells. After washing with 0.05% PBS-Tween, anti-human IgE antibody (1:2000; ThermoFisher) was added to each well and incubated for 1 hour. The wells were washed 5 times with 0.05% PBS-Tween and incubated with HRP conjugated anti-rabbit IgG secondary

antibody (1:2000; Novusbio, Littleton, CO, USA) for 1 hour, followed by incubation with TMB substrate (BD biosciences, Franklin Lakes, NJ, USA) for 10 minutes. The reaction was stopped by the addition of 2N H₂SO₄ and the absorbance values were read by using an ELISA reader (BioTek, Winooski, VT, USA) at 450 nm. The absolute levels of free IgE in each serum were calculated from the standard curve incubated simultaneously. The cutoff point determined by the mean plus 2 times the standard deviation (SD) of the absorbance values of control sera was set as the threshold for high free IgE versus low free IgE level; 1 IU/mL of serum total IgE is equivalent to 2.4 ng/mL of serum free IgE.

ELISA inhibition test

Competitive ELISA inhibition tests were carried out to determine the binding specificity of free IgE to the IgE_{TRAP} protein and to compare between those of omalizumab and anti-human IgE antibody (ThermoFisher). The pooled sera (90 µL) from 2 asthmatic patients with high free IgE levels were preincubated overnight at 4°C, with increasing amounts (0.1 to 10 µg/mL protein concentration) of IgE_{TRAP}, omalizumab (Xolair[®]; Novartis, Basel, Switzerland), and anti-human IgE antibody. Each serum was incubated with the IgE_{TRAP} protein-coated microtiter plates, and the same steps were taken in the ELISA assay as previously described. The sera were preincubated with PBS, instead of inhibitors as control parameter. The percent inhibition was calculated as follows: $100 - (\text{absorbance with inhibitor} / \text{absorbance without inhibitor}) \times 100$.

Statistical analysis

The χ^2 test was used to examine differences in categorical variables, and the *t* test was

applied to determine differences in continuous variables for comparative analyses of the 2 groups. For variables with log-normal distributions, log-transformations were performed. Geometric means and asymmetric SDs were calculated from $\log_{10}$ -scaled mean and SD as follows: geometric mean = $10^{(\text{mean})}$; asymmetric upper SD = $10^{(\text{mean} + \text{SD})} - 10^{(\text{mean})}$; asymmetric lower SD = $10^{(\text{mean} - \text{SD})} - 10^{(\text{mean})}$. Correlations were analyzed with the Pearson correlation coefficient (R). Receiver operating characteristic (ROC) curve analysis was used to evaluate the diagnostic value and to compare utility between serum free IgE and total IgE for assessing atopic status and type 2-high asthma. All statistical analyses were performed using IBM SPSS 25.0 (IBM Corp., Chicago, IL, USA) and MedCalc version 15.5 (MedCalc, Mariakerke, Belgium) statistical software. Levels of significance were presented as P values $<.05$, $<.01$, or $<.001$. Statistical significance was set at $P <.05$.

RESULTS

Clinical characteristics of the study subjects

In total, 116 patients with asthma and 32 healthy control subjects were enrolled in this study. The clinical characteristics of the study subjects are presented in Table 1. Compared to healthy control subjects, asthmatic patients did not differ with respect to mean age or sex proportion. However, as expected, asthmatic patients had higher atopic status ($P = .037$), serum total IgE levels ($P < .001$), and blood eosinophil counts ($P < .001$) as well as had lower forced expiratory volume in 1 second (FEV_1) percent ($P < .001$) and FEV_1 /forced vital capacity (FVC) ratios ($P < .001$) than did healthy controls.

ELISA and IgE-ELISA inhibition test

Figure 1 shows comparison of serum free IgE levels (measured by ELISA) between

asthmatics and healthy controls. Significantly higher levels were noted in asthmatic patients than in healthy controls ($P < .001$; Figure 1, A). A close positive correlation was observed between log-transformed free IgE and total IgE levels ($r = 0.853$, $P < .001$; Figure 1, B). There were no significant differences in the distribution of serum free IgE and total IgE levels in asthmatic and atopic patients. (eFigure 2, A and B). When ELISA inhibition tests were performed in the sera of 2 asthmatic patients with a high serum free IgE level, significant inhibitions were noted with serial additions (0.1-10 $\mu\text{g/mL}$) of the IgE_{TRAP}, omalizumab and anti-human IgE antibody in a dose-dependent manner, demonstrating that greater inhibitions were noted by IgE_{TRAP} than by omalizumab or anti-human IgE antibody (Figure 2, A and B). In addition, the reproducibility of the ELISA was found within 10%.

Associations of serum free IgE levels with type 2 inflammation markers and lung function parameters

Asthmatic patients were classified into the high free IgE group (≥ 220.7 ng/mL, $n = 58$) and the low free IgE group (< 220.7 ng/mL, $n = 58$); the cutoff point was determined from mean + 2SD of serum free IgE for healthy controls ($70.1 + 2 \times 75.3$). Comparisons of patients' characteristics between the 2 groups are summarized in Table 1. Blood eosinophil counts ($P = .026$), serum periostin levels ($P = .010$) and FeNO levels ($P = .038$) as well as serum total IgE levels ($P < .001$) were significantly higher in the high free IgE group than in the low free IgE group. FEV₁(%) values ($P = .022$) and FEV₁/FVC ratios ($P = .090$) were lower in the high free IgE group than in the low free IgE group. In all, 75 patients were able to produce induced sputum, comprising 42 patients in the high free IgE and 33 patients in the low free IgE groups. Sputum eosinophil counts were significantly higher in the high free IgE group than in the low free IgE group ($P = .024$). No significant difference in provocative

concentration of methacholine causing a 20% decrease in FEV₁ was found between the 2 groups ($P = .378$). On the contrary, serum total IgE were not associated with these clinical and laboratory parameters (eTable 1 in the Online Repository).

Comparisons of serum free IgE levels between the 2 subgroups according to type 2 inflammation markers

Subjects with asthma were classified into 2 subgroups each according to blood eosinophil count, sputum eosinophil count and FeNO level: high (≥ 150 / μ L) and low (< 150 / μ L) blood eosinophil subgroups, high ($\geq 2\%$) and low ($< 2\%$) sputum eosinophil subgroups, and high (≥ 20 ppb) and low (< 20 ppb) FeNO subgroups (Figure 3, A, B and C). The cutoff points for determining high and low subgroups were defined according to the GINA guidelines. The serum free IgE levels were significantly higher in subgroups with high levels of individual biomarkers than those with low levels of individual biomarkers (blood eosinophil count, $P = .004$; sputum eosinophil, $P = .003$; FeNO, $P = .029$). When subjects with asthma were divided into the type 2-high and type 2-low groups, as prescribed above, the type 2-high group showed significantly higher levels of serum free IgE than the type 2-low asthma group ($P < .001$; Figure 3, D).

Comparisons of serum free IgE levels according to asthma control status and atopy

When patients with asthma were classified into the controlled and uncontrolled asthma groups according to the GINA-defined asthma control status, the uncontrolled group showed significantly higher levels of serum free IgE than the controlled group ($P < .001$; Figure 3, E). When subjects were classified into the atopic and nonatopic groups according to results of

skin prick test and/or allergen-specific IgE levels, the atopic group had significantly higher serum free IgE levels than the nonatopic group in both asthmatic patients and healthy control subjects ($P < .001$; Figure 3, *F*). There was no significant difference between nonatopic asthmatics and atopic healthy controls ($P = .684$).

Diagnostic value of serum free IgE in predicting atopy and type 2 high asthma

Receiver operating characteristic curves were used to evaluate the potential value of serum free IgE and to determine the cutoff points for identifying atopic status and type 2 high asthma. The diagnostic value of serum free IgE was compared to serum total IgE, a commonly used traditional serological marker. In the ROC analyses of detecting type 2-high asthma, the area under the curve (AUC) of serum free IgE was 0.729 with 71.3% sensitivity and 72.7% specificity at a cutoff point of 120.8 ng/mL, and that of serum total IgE was 0.572 with 73.4% sensitivity and 50.0% specificity at a cutoff point of 91 IU/mL (Figure 4, *A*). A pairwise comparison of ROC curves showed that free IgE had a significantly greater AUC than total IgE ($P < .001$). In the ROC curves for predicting atopic status, the AUC of serum free IgE is 0.759 with 83.3% sensitivity and 59.4% specificity at a cutoff point of 82.8 ng/mL, whereas that of serum total IgE is 0.720 with 77.4% sensitivity and 60.9% specificity at a cutoff point of 92.5 IU/mL (Figure 4, *B*). The AUC of free IgE is higher than that of total IgE, albeit without a statistically significant difference ($P = .078$). Additionally, the AUC of serum free IgE for assessing asthma from healthy controls is 0.810 (68.4% sensitivity and 79.4% specificity at a cutoff point 110.2 ng/mL), demonstrating that free IgE has a significantly higher AUC compared to total IgE ($P = .006$; Figure 4, *C*).

DISCUSSION

Several studies performed in adult asthmatic patients during omalizumab treatment have shown that serum free IgE could provide a useful guidance to select right patients and to monitor therapeutic responses.¹¹⁻¹⁴ To evaluate the clinical relevance of serum free IgE in adult asthmatics, we developed a new ELISA-based assay using a novel IgE_{TRAP} protein. Using this assay, we found that serum free IgE level is a useful serological marker for determining atopic status to differentiate between adult asthmatics and non-atopic healthy controls. The present study demonstrated significant associations between serum free IgE level and type 2 inflammation markers (higher blood/sputum eosinophil counts and higher levels of serum periostin/FeNO/total IgE); asthmatics with type 2 markers had higher free IgE level as well as lower lung function and uncontrolled status of asthma, suggesting that circulating free IgE may affect the severity and control status of asthma. Taken together, high serum free IgE may be a potential biomarker for the diagnosis of type 2-high asthma as well as atopic status in adults. In addition, serum free IgE shows better diagnostic performance compared to serum total IgE in assessing type 2 asthma and atopic status.

It has been shown that IgE plays a role in the regulation of FcεRI expression on mast cells, basophils, and antigen-presenting cells. Interestingly, omalizumab that binds to free IgE in the circulation markedly reduced the density of FcεRI on mast cells, basophils and dendritic cells, concomitantly with the reduction in serum free IgE levels.^{18, 19} A recent study has suggested that CD23 on B cells is also up-regulated by serum IgE according to the rules that guide the expression of FcεRI.⁶ Thus, it is reasonable to postulate that free IgE in the circulation could play a major role in controlling allergic immune responses. The present study demonstrated circulating free IgE levels using the ELISA technique with applying the newly developed hybrid protein, which was found to be a reliable and easy-to-perform method to detect serum free IgE. In addition, the binding specificity and reproducibility

thereof were validated. The results of free IgE were significantly associated with type 2 inflammatory markers including blood/sputum eosinophils, FeNO and total IgE as well as baseline lung function and asthma control status in asthmatic patients. IgE-activated basophils and mast cells lead to the synthesis and release of inflammatory mediators and cytokines, which initiate the late-phase allergic reaction with recruitment and activation of eosinophils. Persistent eosinophilic airway inflammation has been shown to be associated with disease severity and airflow limitation. Eosinophils cause bronchial tissue damage by releasing pro-inflammatory cytokines, chemokines and granule proteins as a consequence, leading to airway remodeling by persistent airway inflammation and repair.²⁰ It was found that treatment with omalizumab reduced not only blood/sputum eosinophils but also tissue eosinophils in bronchial biopsy.²¹⁻²⁴ Also, it has been shown that omalizumab targeting circulating IgE improves asthma symptoms and reduces frequency of acute exacerbations in asthmatic patients. It is conceivable that free IgE, as a key element of this vicious circle, not only maintains chronic eosinophilic inflammation in asthmatic airway but also leads to asthma exacerbation and uncontrolled status of asthma, resulting in airflow limitation and airway remodeling. Taken together, our findings suggest that free IgE could contribute to initiating and maintaining type 2 inflammatory cascades and persistent eosinophil activation in the asthmatic airway; therefore, free IgE is a major therapeutic target in asthmatic patients, especially those with type 2 asthma.

IgE directly activates airway smooth muscle cells that express FcεR1s and CD23 on their surface, and produce extracellular matrix (key factors involved in airway remodeling) leading to their proliferation and contraction.^{21, 25} Periostin, a matricellular protein, is released from airway epithelial cells and fibroblasts, which is up-regulated by IL-4/ IL-13. Periostin has been reported as a biomarker for type 2 inflammation in asthma and could contribute to airway remodeling and fibrosis; therefore, it can be used to predict clinical efficacy after

omalizumab treatment.²⁶ The omalizumab EXTRA study showed a better clinical responses to omalizumab treatment in the periostin-high group than in the periostin-low group.²⁷ In addition, omalizumab, which selectively binds to free IgE, has shown clinical benefits in the reduction in airway wall thickness and improvement in lung function in atopic and nonatopic asthmatics.^{22-24, 28} The present study demonstrated that patients with high free IgE levels had high periostin levels, but lower FEV₁ (%), FEV₁/FVC values than those with lower free IgE levels. Taken together, serum free IgE could contribute to airway remodeling as well as type 2 airway inflammation, contributing to lung function decline in asthmatics.

Several biologics targeting type 2 cytokines, such as IL-4, IL-5, and IL-13 as well as IgE, have recently emerged as promising treatment options for type 2 asthma. Although they have been shown to reduce asthma exacerbation and corticosteroid use not all patients have equal benefits from them because of heterogeneity of asthma.^{29, 30} Given the emergence of various new biologics, questions on the selection of right patients and right biologics remain to be answered. Our results suggest that serum free IgE may be a useful serum biomarker for predicting and monitoring responses to Th2-targeted biologics, especially anti-IgE antibody treatment, although further clinical trials are needed.

This study has 2 limitations. One is that this is a cross-sectional study. Allergen exposure leads to transient increases in serum free IgE level as variable factors, such as pathogens and pollutants, can also influence asthma-related clinical/laboratory variables. However, we recruited all the study subjects who had maintained anti-asthmatic medications (including inhaled corticosteroids and leukotriene modifiers) according to the GINA guideline by asthma specialists in a single tertiary asthma Center; therefore, the data could reflect real-world clinical practice. The other is that although we confirmed the binding specificity and reproducibility of our ELISA method, it needs to be replicated in other cohorts of various

allergic diseases. Further studies are also needed to validate the cut-off values in a completely distinct cohort. Despite these limitations, this is the first study to demonstrate serum free IgE levels in association with type 2 inflammatory markers in adult asthmatics.

In conclusion, high free IgE levels were detected in the sera of adult asthmatics, especially in those with type 2 inflammatory markers. Its diagnostic value in predicting atopy and type 2 asthma was validated.

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FIGURE LEGENDS

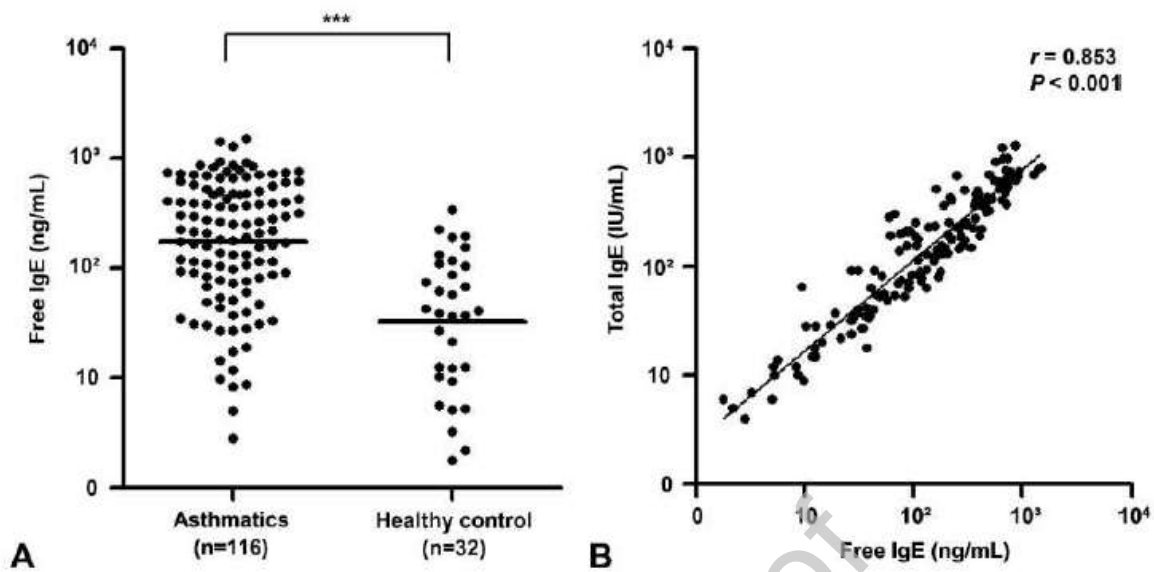


FIGURE 1. Free IgE levels in serum. **(A)** Comparisons of serum free IgE levels in adult asthmatic patients and healthy control subjects. **(B)** Correlation analysis between serum free and total IgE levels. Horizontal bar indicates geometric mean value. * $P < .05$; ** $P < .01$; *** $P < .001$.

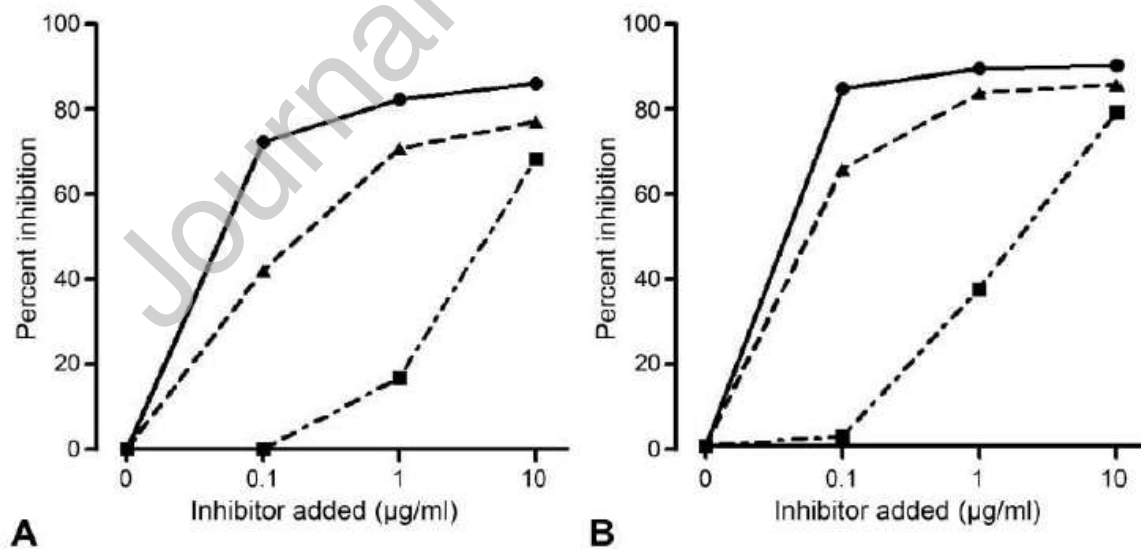


FIGURE 2. Results of competitive ELISA inhibition tests with serial additions of IgE_{TRAP} (●), anti-human IgE antibody (▲), and omalizumab (■) in a dose-dependent manner. **(A)**

and (B) The ELISA inhibition tests are presented from serum of two patients with high free IgE levels. *ELISA*, enzyme-linked immunosorbent assay.

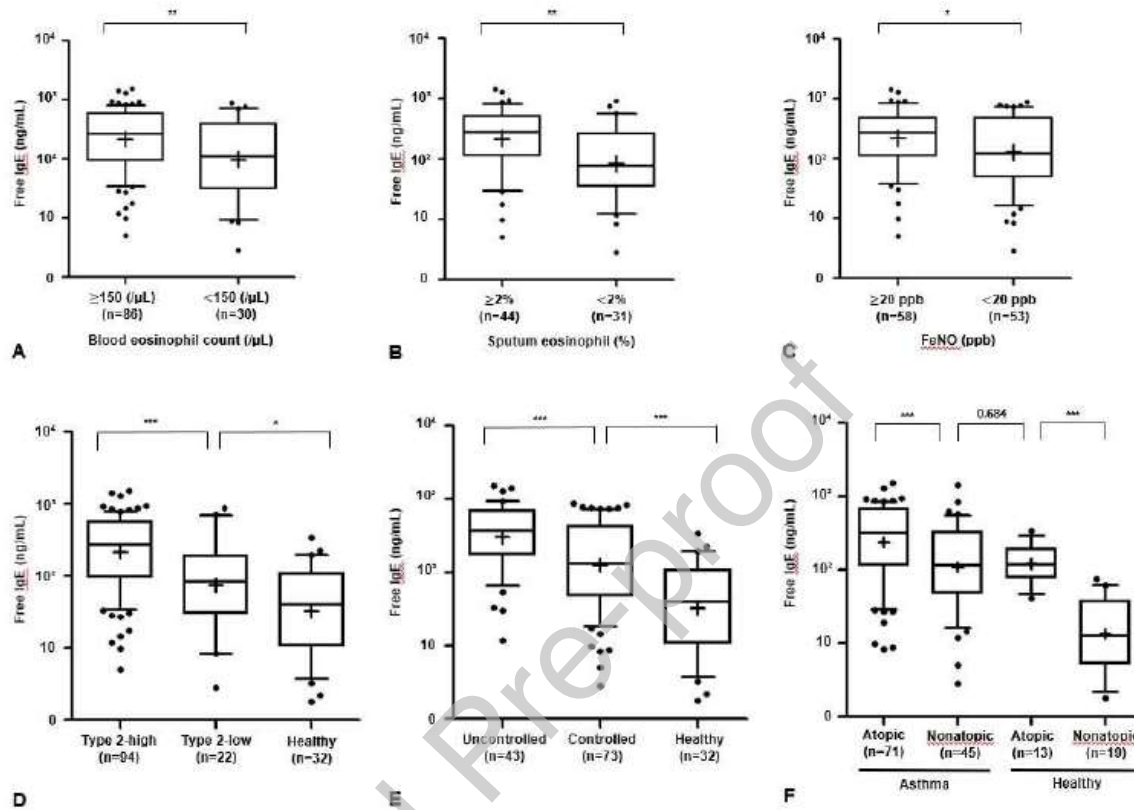


FIGURE 3. Comparisons of serum free IgE levels according to (A) blood eosinophil counts, (B) sputum eosinophils, (C) FeNO levels, (D) type 2-high or low asthma, (E) asthma control status, and (F) atopic status. Box plots span data values between the first and third quartiles with the median line. Crosses represent the geometric mean values. I bars represent the 10 – 90 percentiles, and dots outliers. * $P < .05$; ** $P < .01$; *** $P < .001$.

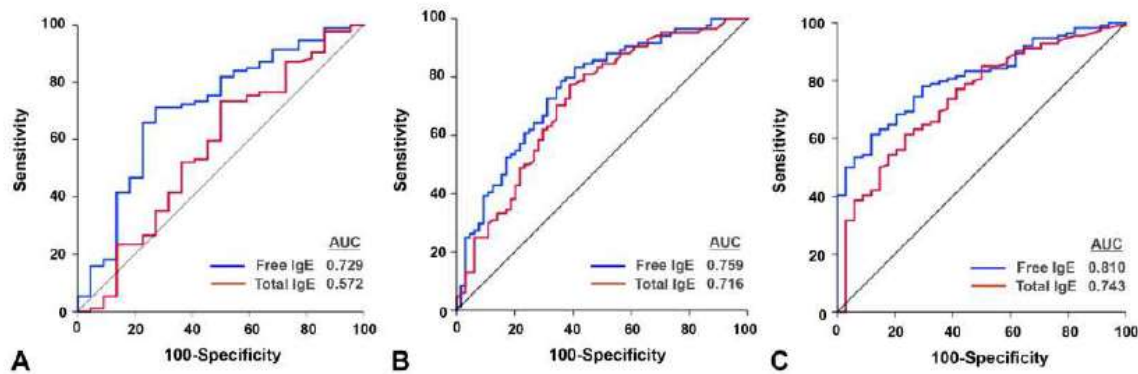


FIGURE 4. Diagnostic utility of serum free and total IgE. **(A)** ROC curves analyses for detecting type 2 asthma from asthmatic patients. **(B)** ROC curves analyses for predicting atopic status from all subjects. **(C)** ROC curves analyses for assessing asthma from all subjects. *AUC*, area under the curve; *ROC*, receiver operating characteristic.

Table I. Clinical characteristics of the study subjects

	Asthmatic patients			Healthy controls (n = 32)	P value	
	Overall (n = 116)	High free IgE (n = 58)	Lower Free IgE (n = 58)		Asthmatics vs controls	high vs low free IgE
Age (yr)	50.0 ± 15.0	49.1 ± 15.8	51.0 ± 14.3	45.0 ± 10.5	.163	.493
Female (%)	79 (68.1)	40 (69.0)	39 (67.2)	20 (62.5)	.551	.842
Atopy (%)	71 (61.2)	44 (75.9)	27 (46.6)	13 (40.6)	.037	<.001
Uncontrolled asthma status (%)	43 (37.1)	29 (50.0)	14 (24.1)	ND	ND	.004
Serum total IgE	173.7	453.0	66.6	51.1	<.001	<.001

(IU/mL)*	+432.6/-	+319.5/-	+114.3/-	+130.3/-		
	124.0	187.4	42.1	36.7		
	238.6	287.7	197.2	118.4		
Blood eosinophil count (/μL)*	+358.4/-	+354.1/-	+334.5/-	+145.0/-	<.001	.026
	143.2	158.7	124.0	65.2		
Serum periostin (ng/mL)	79.6 ± 29.4	86.6 ± 32.9	72.6 ± 23.7	71.8 ± 22.9	.276	.010
Sputum eosinophil (%)*	26.1	31.4	20.9	ND	ND	.024
	+68.6/-18.9	+69.9/-21.7	+64.9/-15.8			
FeNO (ppb)*	22.4	27.1	18.8	20.2	.464	.038
	+34.7/-13.6	+45.2/-16.9	+25.5/-10.8	+14.7/-8.5		
FEV ₁ (%) predicted)	91.6 ± 15.8	88.2 ± 16.1	95.0 ± 15.1	107.3 ± 11.8	<.001	.022
FEV ₁ /FVC (%)	83.0 ± 8.3	81.7 ± 8.8	84.3 ± 7.6	87.3 ± 3.8	<.001	.090
PC ₂₀ level (mg/mL)*	2.7 +10.2/-	2.3 +7.6/-	3.1 +13.4/-	ND	ND	.378
	2.1	1.8	2.5			
Serum free IgE (ng/mL)*	175.2	513.1	59.8	32.5		
	+493.1/-	+310.7/-	+106.2/-	+104.5/-	<.001	ND
	129.3	193.5	38.3	24.8		

Asthmatic patients were classified into the high free IgE group (≥ 220.7 ng/mL) and low free IgE group (< 220.7 ng/mL); the cutoff point was determined by the mean + 2-fold SD of the levels of healthy controls.

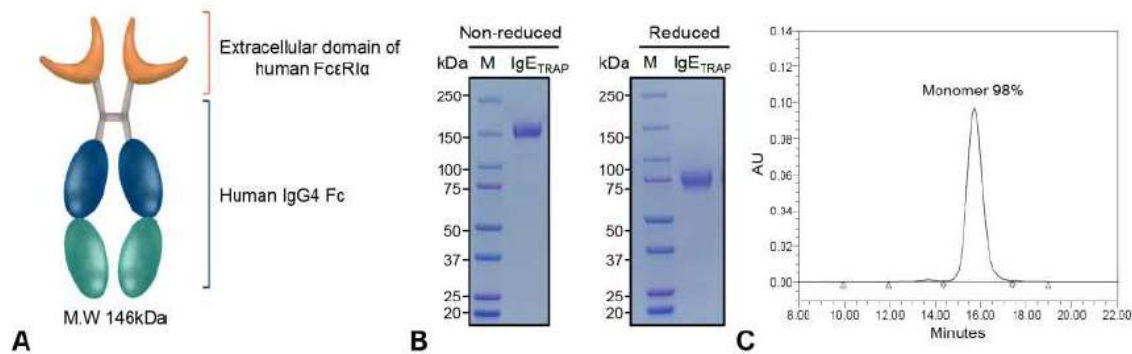
*Geometric mean $\pm$ asymmetric SD. Geometric means and asymmetric SDs were estimated from the log₁₀-scaled means and SDs. *P* values were calculated from log-transformed data.

FeNO, fractional exhaled nitric oxide; *FEV₁*, forced expiratory volume in 1 second; *FVC*, forced vital capacity; *ND*, no data; *PC₂₀*, provocative concentration of methacholine that resulted in a 20% decrease in the *FEV₁*.

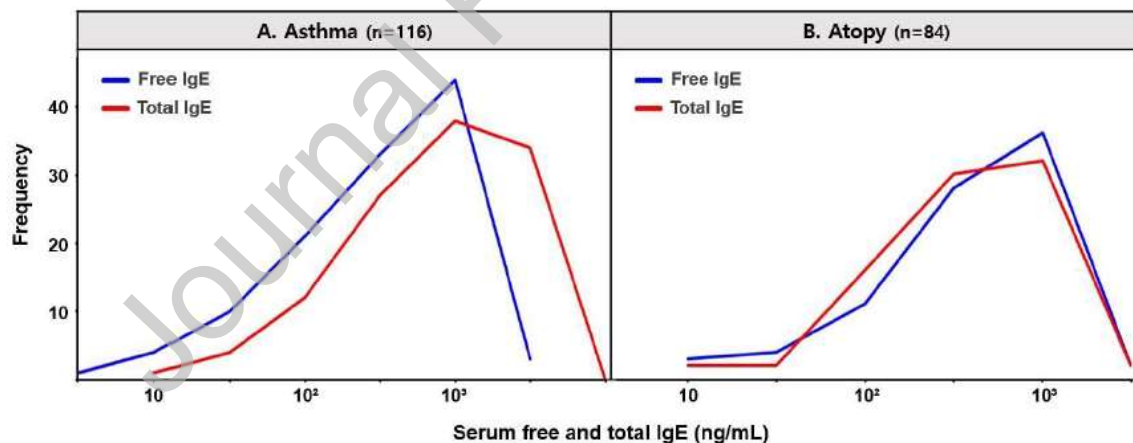
eTable 1. Comparison of clinical characteristics between the high and low serum total IgE groups

	Serum total IgE ≥150 IU/mL (n = 70)	Serum total IgE <150 IU/mL (n = 46)	P value
Age (yr)	49.4 ± 15.4	50.9 ± 14.4	.607
Female (%)	48 (68.6)	31 (67.4)	.894
Atopy (%)	50 (71.4)	21 (45.7)	.005
Uncontrolled asthma status (%)	30 (42.9)	13 (28.3)	.111
Blood eosinophil count (/μL)	364.3 ± 269.0	275.8 ± 231.0	.070
Serum periostin (ng/mL)	84.7 ± 31.2	71.8 ± 24.8	.051
Sputum eosinophil (%)	32.6 ± 34.3	20.0 ± 30.3	.101
FeNO (ppb)	38.7 ± 33.4	26.9 ± 28.5	.064
FEV ₁ (% predicted)	90.2 ± 16.6	93.7 ± 14.6	.247
FEV ₁ /FVC (%)	82.3 ± 9.0	83.9 ± 7.1	.297
PC ₂₀ level (mg/mL)	5.6 ± 7.8	8.8 ± 9.4	.094
Serum free IgE (ng/mL)	496.8 ± 309.1	76.5 ± 60.2	<.001

FeNO, fractional exhaled nitric oxide; *FEV₁*, forced expiratory volume in 1 second; *FVC*, forced vital capacity; *PC₂₀*, provocative concentration of methacholine that resulted in a 20% decrease in the *FEV₁*.



eFigure 1. Structural features of IgE_{TRAP} (A) Schematic diagram of the IgE_{TRAP}. (B) SDS-PAGE analysis of IgE_{TRAP} under non-reducing and reducing conditions. (C) Size exclusion high performance liquid chromatography analysis of IgE_{TRAP}. *SDS-PAGE*, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis.



eFigure 2. Distribution of serum free IgE and total IgE levels in asthmatic patients (A) and in atopic patients (B). The serum IgE values are displayed on log-transformed scales.