ORIGINAL ARTICLE

Serum Specific IgE: A Biomarker of Response to Allergen Immunotherapy

G Ciprandi,[1] M Silvestri[2]

[1]Medicine Department, IRCCS-AOU San Martino, Genoa, Italy
[2]Pediatric Pulmonology and Allergy Unit, Istituto Giannina Gaslini, Genoa, Italy

Abstract

Background: Allergen immunotherapy (AIT) has proven to be effective. However, no biomarkers capable of predicting the clinical response to AIT have been detected. The aim of the present study was to determine a cutoff value for serum specific IgE that could be associated with effective AIT.
Methods: We evaluated 174 allergic patients (83 males) with ages ranging between 6 and 77 years. All patients were monsensitized and received sublingual immunotherapy (SLIT) for at least 3 years with a single allergen extract. Symptom severity was assessed using the visual analog scale (VAS). Drug use was also evaluated. A responder was defined as a patient whose VAS score fell by at least 30% over baseline.
Results: The response to SLIT was considered effective in 145 patients (83.3%). The use of allergen-specific IgE levels >9.74 kU_A/L as a biomarker of effective SLIT yielded a sensitivity value of 96.4%, specificity of 100%, and an area under the receiver operator characteristic curve of 0.987.
Conclusions: Assessment of serum specific-IgE before AIT could be a useful biomarker for predicting response to AIT.

Key words: Allergen-specific immunotherapy. Serum specific IgE. Responder.

Resumen

Antecedentes: La inmunoterapia (IT) Ag-específica es un tratamiento efectivo, pero por el momento no existe un biomarcador capaz de predecir la respuesta clínica a este tratamiento. En este estudio se investiga si un punto de corte en los nivles de IgE específica puede asociarse a la efectividad de la IT.
Métodos: Se incluyeron en este estudio un total de 174 pacientes alérgicos (83 varones) con edades comprendidas entre los 6 y 77 años. Todos ellos estaban tratados con IT al menos durante 3 años frente a un solo extracto antigénico. La gravedad de los síntomas se midió mediante la escala analogica visual (EAV) así como por el consumo de medicamentos. Se consideró a un paciente como respondedor en base a la reducción en la EAV de al menos un 30% comparado con el nivel basal.
Resultados: En cuanto a los resultados obtenidos, la respuesta a SLIT fue considerada como efectiva en 145 (83,3%) de los pacientes. El uso de un punto de corte de IgE específica >9.74 KU/L como biomarcador de la efectividad de la SLIT arrojó una sensibilidad del 96,4% y una especificidad del 100%, con un área bajo la curva ROC de 0.987.
Conclusiones: En conclusión el seguimiento de la IT de la determinación de IgE esp podría ser un biomarcador util para evaluar la respuesta clínica a la IT.

Palabras clave: Inmunoterapia Ag-específica. IgE específica. Biomarcador.

Introduction

Allergen immunotherapy (AIT) is currently the only therapeutic approach to IgE-dependent allergic disorders. Indeed, successful AIT can induce physiologic immune tolerance toward the causal allergen [1].

Double-blind, placebo-controlled trials and meta-analyses have shown AIT to be effective and safe, and indications and contraindication are well defined [2,3]. However, the response to AIT remains a matter of debate, as this approach is not successful in all allergic patients. In addition, no biomarkers have been found to be good predictors of clinical response to AIT.

Allergen-specific IgE can be detected in vivo using skin prick tests or in vitro using quantitative assays.

Changes in some cytokines (IL-4, IL-10, IL-12, IFN-γ, and TGF-β) have been associated with successful AIT, although their measurement is not recommended in clinical practice [4]. A recent study reported that the ratio of serum specific IgE to total IgE could predict the clinical response to AIT in patients monosensitized to grass, *Parietaria judaica, Olea europaea*, and house dust mite (HDM) [5]. However, that study was conducted in a selected cohort of monosensitized allergic patients, and only total IgE was assessed. Daily clinical practice suggests that most allergic patients are polysensitized. In addition, total serum IgE may also be in the normal range in atopic patients and is rarely measured.

Therefore, we performed a retrospective study of candidates for AIT. The aim was to detect a cutoff value for serum specific IgE able to discriminate effective from ineffective AIT.

Materials and Methods

We retrospectively analyzed data for a group of consecutive patients with allergic rhinitis, asthma, or both who were treated with AIT between January 2005 and December 2008 at the Allergy Clinic of Genoa, Genoa, Italy. The local ethics committee approved the study.

All patients underwent sublingual immunotherapy (SLIT) as part of the therapy for allergic rhinitis and/or asthma. The inclusion criteria were as follows: (*a*) documented diagnosis of allergic rhinitis and/or asthma based on patient-reported symptoms, physical examination, and the results of a lung function test (including bronchodilation test); (*b*) documented sensitization (eg, positive skin prick test result); (*c*) clinically relevant allergic symptoms (eg, perceived and bothersome symptoms; (*d*) demonstration of a consistent relationship between inhalation of the sensitizing allergen and occurrence of respiratory symptoms for defining the causal allergen (eg, true allergy); and (*e*) prescription of AIT for a single allergen (eg, in monosensitized patients).

The exclusion criteria were as follows: (*a*) other allergic diseases (eg, atopic dermatitis and eczema); (*b*) clinically relevant anatomic impairment (eg, septum deviation or nasal polyps); (*c*) acute or chronic disorders representing a contraindication to AIT (eg, autoimmune disease and malignancy); and (*d*) allergy to multiple allergens.

Globally, 174 patients (83 males and 91 females) with ages ranging between 6 and 77 years (mean age, 33.9 years; 18 adolescents, and 2 children) were enrolled. All were treated with SLIT for at least 3 years.

Sensitization to the most common classes of aeroallergens was assessed by performing a skin prick test according to the guidelines of the European Academy of Allergy and Clinical Immunology [6]. The allergen panel consisted of the following: HDM (*Dermatophagoides farinae* and *Dermatophagoides pteronyssinus*), cat dander, dog dander, grasses mix, Compositae mix, *P judaica*, birch, hazel, olive, cypress, *Alternaria tenuis, Cladosporium*, and *Aspergillus* mix. The concentration of allergen extracts was 100 immune reactivity units/mL (Stallergènes). A histamine solution in distilled water (10 mg/mL) was used as a positive control, and the glycerol-buffer diluent of the allergen preparations was used as a negative control. Each patient underwent skin testing on the volar surface of the forearm using 1-mm prick lancets (Stallergènes). The skin reaction was recorded after 15 minutes (wheals in both positive and negative controls). A wheal diameter of at least 3 mm was considered a positive reaction.

At the time of diagnosis, serum allergen-specific IgE levels were evaluated in all patients; serum total IgE was assessed in most patients.

A family history of atopy, as well as a history of smoking and onset of respiratory symptoms, was obtained from each patient. Clinical evaluations were performed at baseline and once a year during the 3-year period of SLIT. Evaluations included anterior rhinoscopy and spirometry (in asthmatic patients) and medication use (previous year). We performed a physical examination and administered the visual analog scale (VAS) to assess symptoms and evaluate drug use.

Because SLIT is commercially available and can be prescribed for indications that are recognized both nationally and internationally, our ethics committee required written informed consent for performance of the diagnostic tests and management of clinical data. All patients gave their written informed consent.

The effectiveness of AIT was evaluated considering both clinical improvement and drug use (eg, oral second-generation H1 antihistamines for allergic rhinitis and inhaled short-acting β_2-agonists for asthma symptoms [both prescribed on demand]). Patients globally evaluated both parameters using the VAS. A responder was defined as a patient with a reduction in the VAS score of at least 30% compared with baseline (before starting SLIT).

SLIT (Staloral 300, Stallergenes Italia) was administered for 3 years: preseasonally (4 months) for pollen extracts, continuously for perennial allergens. The maintenance dose was 5 drops administered 3 times a week on alternate days.

Patients were evaluated before starting AIT and at the end of AIT. The pollen season studied was that of the previous year. For HDM, data from the previous year were recorded.

A blood sample was processed at the time of diagnosis and before SLIT. Serum total IgE and allergen-specific IgE levels were determined using an immunofluorimetric assay (ImmunoCAP Thermo Fisher Scientific). Quantitative specific IgE concentrations were expressed in kU_A/L according to the traceable calibration based on the 2nd WHO International Reference Preparation for Human IgE [7]. Specific IgE levels >0.35 kU_A/L were considered positive.

Statistical analysis was performed using the GraphPad software package (GraphPad Prism Software Inc). Normally distributed data were summarized as the arithmetic mean and 95%CI; nonnormally distributed data were expressed as the median (IQR). The Kolmogorov-Smirnov test was used to evaluate the normal distribution. Group comparisons were performed using a 2-sided *t* test or Mann Whitney test when appropriate. Categorical variables between groups were compared using the χ^2 test. The best cutoff for discriminating between responders and nonresponders was estimated on the basis of the receiver operator characteristic (ROC) curve analysis. For all analyses, $P<.05$ was considered statistically significant.

Results

The study population comprised 174 patients (83 males and 91 females), of whom 128 were affected by rhinitis alone, 35 by rhinitis and asthma, and 9 by asthma alone. The mean duration of allergy was 2.7 years (IQR, 1.7-22.4 years). Nasal symptoms were moderate to severe in all patients. Asthma was intermittent in all patients.

Most patients (135) were polysensitized; only 39 were monosensitized. The mean number of allergens in polysenstized patients was 3.2. However, a single allergen was defined in all patients, and the extract of this allergen was used for AIT, as follows: *P judaica*, 32 patients; birch, 45 patients; HDM, 42 patients.

A response to SLIT was recorded in 145 patients (83.3%): 77 were allergic to *P judaica*, 36 to birch, and 32 to HDM. SLIT was considered clinically ineffective in 29 patients (16.7%): 9 were allergic to *P judaica*, 10 to birch, and 10 to HDM.

Table 1 shows the demographic and clinical characteristics of the study population (responders and nonresponders). Serum total and allergen-specific IgE levels were significantly higher in patients with a positive response to SLIT than in patients without a response. Conversely, we did not find any difference in gender distribution, blood eosinophil percentages, and pulmonary function parameters. No relationship was detected between allergen dose, total dose received, and clinical response.

When patients with rhinitis only were analyzed, those with effective clinical responses to SLIT had higher levels of serum total IgE and serum allergen-specific IgE than patients with an ineffective response (data not shown).

Serum total IgE and allergen-specific IgE were not affected by age.

On the basis of the ROC curve analysis, we identified 130 kU_A/L as the optimal cutoff value of serum total IgE for discriminating effective from ineffective clinical response to SLIT (Figure 1A) with a sensitivity of 69.2% (95%CI, 59.4%-77.9%), specificity of 63.2% (95%CI, 38.4%-83.6%), positive predictive value (PPV) of 91.0% (95%CI, 84.0%-97.0%), negative predictive value (NPV) of (27.0%) (95%CI, 15.0%-43.0%), and

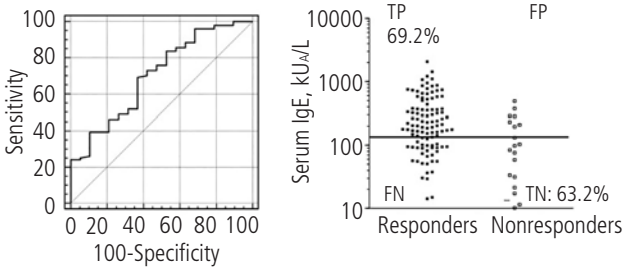

Figure 1. A, Receiver operator characteristic curve analysis to determine the optimal cut point at which serum total IgE level discriminates between patients with an effective clinical response to SLIT and patients with an ineffective response to SLIT. B, Ability of the chosen cut point of 130 kU_A/L to discriminate between patients with and without an effective response to SLIT. TP indicates true positive; FP, false positive; FN, false negative; TN, true negative.

Table 1. Demographic and Clinical Characteristics in Responders and Nonresponders[a]

Variables	Whole population (N=174)	Responders (n=145)	Nonresponders (n=29)	*P* Value
Male gender, No (%)	83 (47.4)	72 (50.3)	10 (35.7)	.19
Age, y, median (IQR)	34.0 (25.0-45.0)	33.0 (25.0-45.0)	37.5 (27.5-45.5)	.40
Blood eosinophils, %	4.1 (2.8-6.0)	4.1 (3.0-6.2)	3.5 (2.1-5.1)	.31
Serum total IgE, kU_A/L	190.0 (92.0-365.0)	202.5 (98.0-401.0)	97.0 (31.0-229.0)	.004
Allergen-specific IgE, kU_A/L				
Specific IgE to HDM, kU_A/L	29.8 (9.5-72.3)	16.8 (11.8-28.6)	2.5 (0.6-4.4)	<.0001
Specific IgE to *Parietaria*, kU_A/L	28.3 (14.9-55.7)	34.0 (19.4-69.3)	6.0 (5.3-6.8)	<.0001
Specific IgE to birch, kU_A/L	34.0 (10.4-58.6)	43.9 (20.4-72.4)	5.5 (4.5-8.9)	<.0001
FEV_1, % predicted	98.9 (11.6)	98.5 (11.3)	101.2 (13.6)	.61
FVC, % predicted	103.5 (13.9)	103.6 (14.1)	103.0 (14.5)	.93
FEV_1/FVC, % predicted	81.3 (5.3)	81.5 (5.2)	80.4 (6.1)	.67
FEF_{25-75}, % predicted	78.6 (15.4)	79.6 (15.0)	73.7 (18.0)	.40

Abbreviations: FEF_{25-75}, forced expiratory flow, midexpiratory phase; FEV_1, forced expiratory volume in 1 second; FVC, forced vital capacity; HDM, house dust mite.
[a]All data are presented as median (IQR), with the exception of pulmonary function parameters, which are reported as mean (SD).

AUC of 0.705 (95%CI, 0.617-0.784). The ability of this chosen cutoff of 130 kU_A/L to discriminate between patients with and without an effective response to SLIT is illustrated in Figure 1B.

From a more practical point of view, the positive and negative likelihood ratios were 1.88 and 0.49, giving a statistically significant diagnostic odds ratio of 3.84. This means that the probability of having an effective response to SLIT is about 4-fold higher in patients with serum total IgE levels >130 kU_A/L than in those with lower levels.

Allergen-specific IgE levels >9.74 kU_A/L allowed detection of effective SLIT with a sensitivity of 96.5% (95%CI, 92.1%-98.9%), a specificity of 100.0% (95%CI, 100.0%-100.0%), a PPV of 100% (95%CI, 97.0%-100.0%), an NPV of 85% (95%CI, 68.0%-95.0%), and an AUC of 0.987 (95%CI, 0.957-0.998) (Figure 2A). The ability of this chosen cutoff to discriminate between patients with and without an effective response to SLIT is illustrated in Figure 2B.

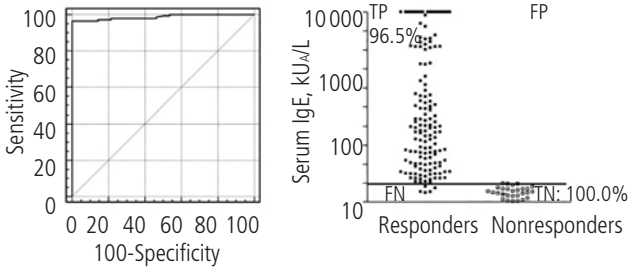

Figure 2. A, Receiver operator characteristic curve analysis to determine the optimal cut point at which serum allergen-specific IgE level discriminates between patients with an effective clinical response to SLIT and an ineffective response to SLIT. B, Ability of the chosen cut point of 9.74 kU_A/L to discriminate between patients with and without an effective response to SLIT. TP indicates true positive; FP, false positive; FN, false negative; TN, true negative.

Comparing ROC curves for serum total and allergen-specific IgE levels, we found a statistically significant difference ($P<.0001$) between AUCs, suggesting that allergen-specific IgE levels may better predict effective SLIT (data not shown).

Table 2 shows the best cutoff values of IgE levels specific for each allergen, as well as the sensitivity, specificity, PPV, and NPV for each test used to predict the clinical response to allergen immunotherapy.

Discussion

Our retrospective study demonstrated that measurement of serum specific IgE before starting SLIT could help to identify patients who respond to AIT. In fact, a specific cutoff was defined for each allergen, as follows: HDM, >9.5 kU_A/L; birch, >9.74 kU_A/L; and *Parietaria*, >8.04 kU_A/L.

The availability of a biomarker capable of predicting the outcome of AIT would be extremely useful in clinical practice, although it should be reliable and easily applicable. The serum specific-IgE assay is a popular test with allergists. Moreover, positive IgE is a typical marker for the allergic phenotype, and high levels of serum specific IgE may be associated with symptom severity [8]. The present study, conducted in a real-life setting, confirmed the results of a previous study of monosensitized patients [5]. Of note, most patients seen in daily practice are polysensitized [9], and this may hamper prescription of AIT for some physicians. However, given that a thorough diagnostic workup enables us to define the causal allergen in most patients, AIT can be prescribed correctly [9]. In this regard, the present study shows that even though most patients were polysensitized, a single allergen extract could be prescribed in all patients. Therefore, a clinically relevant outcome here is that prescription of AIT should be preceded by a careful analysis of sensitization profile and clinical history.

In addition, our findings demonstrate that assessment of serum specific IgE may be predictive of the response to AIT without the need to assess total IgE.

Nevertheless, as our study was retrospective and not adequately controlled, it should be followed by rigorous prospective trials in larger cohorts to confirm the findings.

In conclusion, determination of serum specific-IgE before SLIT might be considered a useful biomarker for predicting the response to AIT.

Acknowledgments

We are grateful to GW Canonica and G Passalacqua for stimulating comments.

Table 2. Sensitivity, Specificity, and Positive and Negative Predictive Values for Each Test Used to Predict the Clinical Response to Specific Immunotherapy

	AUC	Sensitivity	Specificity	PPV	PV
Positive test to predict the clinical response to SIT					
HDM-specific IgE >9.50 kU_A/L	99.4 (90.3-1.00)	96.9 (83.7-99.5)	100.0 (100.0-100.0)	100.0 (89.0-100.0)	90.9 (58.7-99.8)
Bet-specific IgE >9.74 kU_A/L	97.5 (87.8-99.6)	94.4 (81.3-99.2)	100.0 (100.0-100.0)	100.0 (89.7-100.0)	81.8 (48.2-97.7)
Par-specific IgE >8.04 kU_A/L	98.9 (93.7.-99.8)	97.4 (90.8-99.6)	100.0 (100.0-100.0)	100.0 (95.1-100.0)	81.8 (48.2-97.7)

Abbreviations: AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value.

Funding

The authors declare that no funding was received for the present study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

1. Jutel M, Akdis CA. Immunological mechanisms of allergen-specific immunotherapy. Allergy. 2011;66:725-32.
2. Canonica GW, Bousquet J, Casale T, Lockey RF, Baena-Cagnani CE, Pawankar R, Potter PC, Bousquet PJ, Cox LS, Durham SR, Nelson HS, Passalacqua G, Ryan DP, Brozek JL, Compalati E, Dahl R, Delgado L, van Wijk RG, Gower RG, Ledford DK, Filho NR, Valovirta EJ, Yusuf OM, Zuberbier T, Akhanda W, Almarales RC, Ansotegui I, Bonifazi F, Ceuppens J, Chivato T, Dimova D, Dumitrascu D, Fontana L, Katelaris CH, Kaulsay R, Kuna P, Larenas-Linnemann D, Manoussakis M, Nekam K, Nunes C, O'Hehir R, Olaguibel JM, Onder NB, Park JW, Priftanji A, Puy R, Sarmiento L, Scadding G, Schmid-Grendelmeier P, Seberova E, Sepiashvili R, Solé D, Togias A, Tomino C, Toskala E, Van Beever H, Vieths S. Sublingual immunotherapy: World Allergy Organization Position Paper 2009. Allergy. 2009;64(Suppl 91):1-59.
3. Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. J Allergy Clin Immunol. 1998;102:558-62
4. Vitaliti G, Leonardi S, Miraglia del Giudice M, Artusio L, Caimmi D, Salpietro A, Arrigo T, Salpietro C, Ciprandi G, La Rosa M. Mucosal immunity and sublingual immunotherapy in respiratory disorders. J Biol Regulator. 2012;26:85-94.
5. Di Lorenzo G, Mansueto P, Pacor ML, Rizzo M, Castello F, Martinelli N, Ditta V, Lo Bianco C, Leto-Barone MS, D'Alcamo A, Di Fede G, Rini GB, Ditto AM. Evaluation of serum s-IgE/total IgE ratio in predicting clinical response to allergen specific immunotherapy. J Allergy Clin Immunol. 2009;123:1103-10.
6. Dreborg S (Ed.). EAACI Subcommittee on Skin Tests. Skin tests used in type I allergy testing. Position Paper. Allergy. 1989;44(Suppl 10):22-31.
7. www.nibsc.org/documents/ifu/75-502.pdf
8. Rolinck-Werninghaus C, Keil T, Kopp M, Zielen S, Schaurer U, von Berg A, Wahn U, Hamelmann E; Omalizumab Rhinitis Study Group. Specific IgE serum concentration is associated with symptom severity in children with seasonal allergic rhinitis. Allergy. 2008;63:1339-44.
9. Ciprandi G, Incorvaia C, Puccinelli P, Soffia S, Scurati S, Frati F. The polysensitization as a challenge for the allergist: the suggestions provided by the Polysensitization Impact on Allergen Immunotherapy studies. Expert Opin Biol Ther. 2011;11:715-22.

Manuscript received February 20, 2013; accepted for publication April 30, 2013.

Giorgio Ciprandi

Viale Benedetto XV 6
16132 Genoa, Italy
E-mail: gio.cip@libero.it