Evaluation of serum s-IgE/total IgE ratio in predicting clinical response to allergen-specific immunotherapy

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Background: To date, no predictive tests for the clinical response to allergen-specific immunotherapy (ASI) are available. Therefore an *in vivo* or *in vitro* test would be of great value.
Objective: We sought to evaluate pretreatment parameters used in diagnosing allergic rhinitis and determining serum specific IgE (s-IgE) levels, serum total IgE (t-IgE) levels, and blood eosinophil counts and to identify whether can be used to predict clinical improvement in monosensitized patients with allergic rhinitis with or without asthma treated with immunotherapy.
Methods: We analyzed 279 patients who had undergone 4 years of ASI administered either by means of the subcutaneous immunotherapy (76 patients) or sublingual immunotherapy (203 patients) routes. Serum t-IgE and s-IgE levels, blood eosinophil counts, and serum s-IgE/t-IgE ratios were calculated and tested for correlation with clinical response to ASI. Receiver operating characteristic curves were determined. Predicted probabilities and predictive areas under the curve were calculated.
Results: The clinical response to ASI was effective in 145 (52.0%) of 279 total patients, 42 (55.2%) of 76 patients treated with subcutaneous immunotherapy, and 103 (50.7%) of 203 patients treated with sublingual immunotherapy. A significant correlation was found between the serum s-IgE/t-IgE ratio and the clinical response to ASI, with high ratios (>16.2) associated with an effective response. The sensitivity and specificity of the area under the curve of the ratio were higher than those of serum s-IgE and t-IgE alone.
Conclusion: The calculation of the serum s-IgE/t-IgE ratio for predicting the clinical response to ASI offers an advantage over measuring t-IgE and s-IgE levels in monosensitized patients for the following allergens: grass, *Parietaria judaica*,

Olea europea, and house dust mite. (J Allergy Clin Immunol 2009;123:1103-10.)

Allergen-specific immunotherapy (ASI) is the practice of administering gradually increasing doses of allergens (allergen extracts or vaccines) to reduce allergic symptoms and the need for medication resulting from exposure to a specific allergen.[1,2] Subcutaneous immunotherapy (SCIT) was introduced into clinical practice early in the 20th century.[3] In 1986, sublingual immunotherapy (SLIT) was introduced.[4,5] Double-blind placebo-controlled trials and meta-analyses confirm the efficacy, safety, and indications and contraindications of both SCIT and SLIT.[6-8] However, the efficacy of ASI is still debated, despite solid documentation. ASI, in practice, is indicated for patients for whom the causative role of the allergen is well documented. This is determined most commonly *in vivo* in skin tests (eg, skin prick testing) and *in vitro* in quantitative assays for serum specific IgE (s-IgE; eg, Unicap 100; Phadia, Uppsala, Sweden). It is unclear how serum total IgE (t-IgE) measurements, alone or in relation to s-IgE measurements, or blood eosinophil (b-eos) counts should be interpreted, as well as what role they should play in selecting patients for ASI in clinical practice. Furthermore, the significance of serum s-IgE levels might vary depending on the level of t-IgE.

In this study we report the clinical results of 279 patients who received 4 years of ASI in a clinical setting administered either by means of SCIT or SLIT. We retrospectively examined the relationship of the following parameters determined at the time of diagnosis: diameter of wheal induced by the allergen, serum t-IgE levels, serum s-IgE levels, b-eos counts, and clinical response to ASI. We used receiver operating characteristic (ROC) curves to determine the sensitivity, specificity, and predicted values for wheal diameter, serum s-IgE level, serum t-IgE level, serum s-IgE/t-IgE ratio, and b-eos count, all obtained at the time of diagnosis, in predicting one's response to ASI.

METHODS
Patients

We retrospectively analyzed monosensitized adult patients consecutively referred to the Outpatient Allergy Units of the Dipartimento di Medicina Clinica e delle Patologie Emergenti of the University of Palermo, Italy, and of the Dipartimento di Medicina Clinica e Medicina Sperimentale of the University of Verona, Italy, between January 1995 and December 2000 for evaluation of their allergic rhinitis with or without asthma symptoms. All patients underwent ASI as part of the therapy for allergic rhinitis. Patients did

not have other allergic diseases, such as atopic dermatitis, eczema, or nasal polyps.

A total of 279 subjects (120 male and 159 female subjects), with ages ranging between 18 and 56 years (mean age, 29.5 ± 8.1 years) were selected: 126 subjects from Palermo and 153 subjects from Verona, respectively.

All patients presented with a clinical diagnosis of allergic rhinitis with or without asthma based on patient-reported symptoms, physical examination, and a normal baseline lung function test (baseline FEV_1 ≥80% of predictive value). All patients has positive skin test responses for 1 of 11 common airborne allergens, as determined by using the skin prick test method, with monosensitization to 1 of the following: grass, *Parietaria judaica*, *Olea europea*, or house dust mite (HDM). All were treated with ASI for at least 4 years administered either as SCIT or SLIT. At the time of diagnosis, blood was collected for analysis of serum t-IgE levels, s-IgE levels, and b-eos counts. Family history of atopy, as well as history of smoking and onset of respiratory symptoms, was obtained from each patient. Clinical evaluations were done at baseline and once a year for the 4-year period of ASI. Evaluations included rhinoscopy and spirometry (if asthmatic), symptom scoring with the visual analog scale (VAS), and medication use, referring to the previous year. Evaluations were conducted at the end of the season for the 3 outdoor allergens and at the end of February for *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*. More precisely, we performed a clinical evaluation and administered the VAS for symptoms in June for patients allergic to grass and *O europea* and in September for patients allergic to *P judaica*. For patients allergic to *D pteronyssinus* and *D farinae*, we performed a clinical evaluation and administered the VAS (in February) because the symptoms were worse in autumn, after vacations, and during winter (for more information, see the Methods section in this article's Online Repository at www.jacionline.org).

Because ASI is commercially available and was prescribed for indications that are recognized both nationally and internationally, our ethics committees required written informed consent for the diagnostic tests only, and this was obtained from each patient.[8-10]

Immunotherapy intervention

The route of administration of ASI (SCIT or SLIT) was chosen by the patient to ensure compliance. Each patient received the maximum tolerated dose, per the manufacturers' recommendations, for both SCIT and SLIT. All the patients in this study tolerated the maximum dose indicated by the manufacturers' recommendations as follows. For SCIT, the maintenance dose was 0.8 mL, which corresponds to 1.6 μg of the major allergen of grass, 0.4 μg of the major allergen of *P judaica*, 9.6 μg of the major allergen of *O europea*, and 3.84 μg of the major allergens of *D pteronyssinus* and *D farinae*. On the other hand, for SLIT, the maintenance dose was 60 drops (1 drop = 25 μL) administered 3 times a week on alternate days, which corresponds to 28.2 μg of the major allergen of grass, 6.6 μg of the major allergen of *P judaica*, 169.2 μg of the major allergen of *O europea*, and 67 μg of the major allergens of *D pteronyssinus* and *D farinae*. These doses were administered monthly. Immunotherapy with grass, *P judaica*, and *O europea* pollen was not interrupted during the spring, but the dose was halved (see the Methods section in this article's Online Repository).

TABLE I. Characteristics of cohort study patients examined in the 2 centers

No. of patients	279
Age (y)*	29.5 (28.6-30.5)
Male/female sex	120/159
SCIT/SLIT	76/203
Atopic family history (yes/no)	116/163
Passive smoking (yes/no)	123/156
Rhinitis/rhinitis plus asthma	152/127
Onset of symptoms (y)*	7.1 (6.4-7.7)
Pollens/HDM sensitization (no)	106/173
Response to ASI (effective/ineffective)	145/134
SPT to allergen (mm)†	5.4 (5.3-5.5)
Serum t-IgE level (kU/L)†	139.3 (121.4-159.9)
Serum s-IgE level (kAU/L)†	20.2 (17.6-23.0)
b-eos Counts (cells × 10^{-3} μL)†	0.35 (0.34-0.37)
Serum s-IgE/t-IgE* ratio	14.4 (12.3-16.9)

*Mean (95% CI).
†Geometric mean after logarithmic transformation (95% CI).

Assessment of symptoms and medication use

The effectiveness of ASI was evaluated on the basis of clinical response (reduction in nasal and pulmonary symptoms) and reduction of the pharmacotherapy taken on an as-needed basis (eg, oral second-generation H_1-antihistamine for rhinitis and inhaled short-acting β_2-agonist for asthma symptoms).[10,11] Patients were not previously treated with nasal or inhaled corticosteroids.

VAS. To evaluate symptom response to ASI and reduction of pharmacotherapy, we used a VAS sufficiently sensitive to detect changes in symptom severity (see the Methods section in this article's Online Repository).[12,13]

The response to ASI was determined clinically by asking the patient the following: "Do you feel better than you did before therapy?" The answer could be one of the following:

1. I have not noticed any improvement since I have used ASI.
2. I noticed a worsening of symptoms since I have used ASI.
3. I noticed that my symptoms improved since I have used ASI.

When we analyzed the 1624 ASI VAS responses throughout the 4-year study, we noticed that patients who stated improvement of symptoms during immunotherapy indicated a reduction in their rhinitis (or asthma) VAS values of at least 30% compared with their baseline VAS values before starting immunotherapy at the time of diagnosis. To be sure of the results, for each subject, we used all the ASI values obtained for each year, calculated the mean, and then subtracted the baseline VAS (VASb) from the mean of the VAS obtained over 4 years. Then we divided the result by the baseline VAS value and multiplied by 100, as shown by the following formula:

$$VAS\ decrease\ (\%) = \frac{(Mean\ VAS - VASb)}{VASb} \times 100$$

We evaluated the ASI as effective if the mean of the VAS scores for the 4 years showed a 30% decrease with respect to the value of the VAS score before ASI by using the formula reported above and if the values of the rescue medications indicated at the third and fourth years of ASI were 0 or 1. In all other cases, ASI was evaluated as ineffective. Symptom scoring and the medication used were reviewed by a physician (PM for Palermo and NM for Verona).

Spirometry. FEV_1 and forced expiratory vital capacity were measured with a Gould 2400 automated system (Sensormedics BV, Bilthoven, Netherlands) by using the highest of 3 successive measurements, provided that the difference between measurements was within 100 mL. Spirometric results were followed each year for 4 years. There was no significant change in FEV_1 or forced expiratory vital capacity (all >80% of predicted value) in any patient, irrespective of whether the patient responded clinically to ASI.

TABLE II. Characteristics of the cohort study patients in respect to effective or ineffective clinical response to ASI

	All patients (n = 279)			Patients treated with SCIT (n = 76)			Patients treated with SLIT (n = 203)		
	Effective clinical response	Ineffective clinical response	P value	Effective clinical response	Ineffective clinical response	P value	Effective clinical response	Ineffective clinical response	P value
Patients, no. (%)	145 (52.0)	134 (48.0)		42 (55.2)	34 (44.8)		103 (50.7)	100 (49.3)	
Age (y)*	30.3 (28.9-31.7)	28.7 (27.4-30.1)	.1	29.8 (26.9-32.7)	29.6 (26.7-32.5)	.9	30.5 (28.9-32.0)	28.5 (26.9-30.0)	.06
Male/Female sex	52/93	68/66	.01	11/31	19/15	.01	41/62	49/51	.2
Male (%)	35.8	50.7		26.2	55.9		39.8	49.0	
Female (%)	64.1	49.2		73.8	44.1		60.2	51.0	
SCIT/SLIT	42/103	34/100	.5	NA	NA	NA	NA	NA	NA
SCIT group (%)	28.9	25.3							
SLIT group (%)	70.1	74.7							
Atopic family history (yes/no)	64/81	52/82	.4	36/6	22/12	.06	28/75	30/70	.7
Positive family history (%) group	44.1	38.8		85.7	64.7		27.2	30.0	
Negative family history group (%)	55.9	61.2		14.3	35.3		72.8	70.0	
Passive smoking (yes/no)	65/80	58/76	.8	16/26	14/20	.9	49/54	44/56	.7
Positive smoking group (%)	44.8	43.3		38.1	41.2		47.6	44.0	
Negative smoking group (%)	55.2	56.7		61.9	58.8		52.4	56.0	
Rhinitis/rhinitis plus asthma	72/73	80/54	.1	22/20	20/14	.7	50/53	60/40	.1
Rhinitis group (%)	49.7	59.7		52.4	58.8		48.5	60.0	
Rhinitis-asthma group (%)	50.3	40.3		47.6	41.2		51.5	40.0	
Onset of symptoms (y)*	6.6 (5.8-7.5)	7.5 (6.5-8.4)	.2	7.1 (5.4-8.7)	8.9 (6.6-11.2)	.1	6.5 (5.5-7.5)	7.0 (5.9-8.0)	.4
Pollens/HDM sensitization	97/48	76/58	.1	24/18	15/19	.3	73/30	61/39	.1
Pollens group (%)	66.9	56.7		57.1	44.1		70.9	61.0	
Grass, no. (%)	4 (2.7)	12 (8.9)		1 (2.4)	2 (5.9)		3 (2.9)	10 (10.0)	
P judaica, no. (%)	90 (62.1)	45 (33.6)		23 (54.7)	11 (32.3)		67 (65.1)	34 (34.0)	
O europea, no. (%)	3 (2.1)	19 (14.2)		0 (0)	2 (5.9)		3 (2.9)	17 (17.0)	
HDM, no. (%)	48 (33.1)	58 (43.3)		18 (42.9)	19 (55.9)		30 (29.1)	39 (39.0)	
SPT response to allergen (mm)†	5.4 (5.2-5.6)	5.4 (5.2-5.7)	.9	5.4 (5.1-5.8)	5.3 (4.9-5.7)	.9	5.5 (5.3-5.7)	5.4 (5.2-5.6)	.9
Serum t-IgE level (kU/L)†	84.2 (72.7-97.7)	240.2 (196.2-294.2)	<.0001	105.2 (80.8-137.1)	266.7 (177.4-401.0)	.0007	76.9 (64.3-91.9)	231.8 (182.9-293.9)	<.0001
Serum s-IgE level (kAU/L)†	30.5 (26.1-35.7)	12.9 (10.6-15.6)	<.0001	38.5 (28.6-51.9)	19.3 (12.7-29.2)	.01	27.7 (23.1-33.3)	11.2 (9.1-13.9)	<.0001
b-eos Counts (cells × 10^{-3} μL)†	0.31 (0.29-0.32)	0.46 (0.42-0.49)	<.0001	0.30 (0.27-0.34)	0.46 (0.39-0.53)	.0006	0.31 (0.29-0.34)	0.45 (0.42-0.49)	<.0001
Serum s-IgE/t-IgE* ratio	36.2 (33.3-39.5)	5.4 (4.4-6.6)	<.0001	36.6 (29.6-45.2)	7.2 (5.6-9.4)	<.0001	36.1 (33.1-39.3)	4.8 (3.7-6.2)	<.0001
Patient no. (%) with s-IgE/t-IgE ratio >16.2%	141 (97.2)	17 (12.7)	<.0001	41 (97.6)	2 (5.9)	<.0001	100 (97.1)	15 (15.0)	<.0001
Patient no. (%) with s-IgE/t-IgE ratio ≤16.2%	4 (2.8)	117 (87.3)		1 (2.4)	32 (94.1)		3 (2.9)	85 (85.6)	

NA, Not applicable.
*Mean (95% CI).
†Geometric mean after logarithmic transformation (95% CI).

Skin prick tests. Skin prick tests with a standard aeroallergen panel (Alk-Abelló, Milan, Italy) were performed and evaluated on the volar aspect of the forearm after withholding antihistamines for at least 5 days. The panel included the following extracts: pollens (grass [*Phleum pratense*], mugwort [*Artemisia vulgaris*], pellitory of the wall or sticky weed [*P judaica*], and trees [*O europea* and *Cupressus species*]), HDM (*D pteronyssinus* and *D farinae*), molds (*Alternaria alternata* and *Aspergillus fumigatus*), and animal dander (cat and dog), as well as a negative control (glycerinated saline) and a positive control (histamine, 10 mg/mL). A positive response was defined as any wheal with a diameter 3 mm larger than that elicited by the negative control 15 minutes after application.[14] The wheal diameters were reported for each patient.

TABLE III. Characteristics of patients with effective and ineffective responses to ASI in respect to symptoms

	Patients with rhinitis (n = 152)			Patients with rhinitis plus asthma (n = 127)		
	Effective clinical response	Ineffective clinical response	*P* value	Effective clinical response	Ineffective clinical response	*P* value
Patients, no. (%)	72 (47.4)	80 (52.6)		73 (57.5)	54 (42.5)	
Age (y)*	29.3 (27.5-31.2)	28.3 (26.6-30.0)	.4	31.3 (29.2-33.3)	29.4 (27.2-31.6)	.2
Male/female sex	25/47	41/39	.04	27/46	27/27	.1
Male (%)	34.7	51.2		37.0	50.0	
Female (%)	65.3	48.8		63.0	50.0	
SCIT/SLIT	22/50	20/60	.4	20/53	14/40	.9
SCIT group (%)	30.6	25.0		27.4	26.0	
SLIT group (%)	69.4	75.0		72.6	74.0	
Atopic family history (yes/no)	36/36	34/46	.4	28/45	18/36	.5
Positive family history group (%)	50.0	42.5		38.4	33.3	
Negative family history group (%)	40.0	57.5		61.6	66.7	
Passive smoking (yes/no)	30/42	33/47	.9	35/38	25/29	.8
Positive smoking group (%)	41.7	41.2		48.0	46.3	
Negative smoking group (%)	58.3	58.8		52.0	53.7	
Onset of symptoms (y)†	5.1 (4.2-6.0)	7.2 (5.9-8.5)	.01	8.1 (6.8-9.5)	7.9 (6.4-9.4)	.5
Pollens/HDM sensitization	45/27	46/34	.6	52/21	30/24	.1
Pollens group (%)	62.5	57.5		71.2	55.6	
HDM group (%)	37.5	42.5		28.8	44.4	
Grass, no. (%)	1 (1.4)	8 (10)		3 (4.1)	4 (7.4)	
P judaica, no. (%)	43 (59.7)	24 (30)		47 (64.4)	21 (38.9)	
O europea, no. (%)	1 (1.4)	14 (17)		2 (2.7)	5 (9.3)	
HDM, no. (%)	27 (37.5)	34 (43)		21 (28.8)	24 (44.4)	
SPT response to allergen (mm)†	5.3 (5.0-5.6)	5.3 (5.1-5.6)	.7	5.4 (5.1-5.6)	5.3 (5.0-5.6)	.8
Serum t-IgE level (kU/L)†	84.2 (68.7-103.2)	206.4 (161.5-263.8)	<.0001	84.2 (67.6-104.9)	300.8 (212.3-428.2)	<.0001
Serum s-IgE level (kAU/L)†	30.1 (24.6-37.5)	10.0 (7.9-12.7)	<.0001	30.9 (24.6-38.7)	18.6 (13.6-25.3)	.01
b-eos Counts (cells × 10^{-3} μL)†	0.31 (0.29-0.33)	0.40 (0.37-0.42)	<.0001	0.30 (0.28-0.33)	0.43 (0.40-0.47)	<.0001
Serum s-IgE/t-IgE* ratio	41.5 (36.9-48.1)	9.9 (7.6-12.2)	<.0001	39.8 (35.1-44.5)	8.1 (8.5-9.7)	<.0001
Patient no. (%) with s-IgE/t-IgE ratio >16.2%	70 (97.2)	10 (12.5)	<.0001	71 (97.3)	7 (13.0)	<.0001
Patient no. (%) with s-IgE/t-IgE ratio ≤16.2%	2 (2.8)	70 (87.5)		2 (2.7)	47 (87.0)	

*Mean (95% CI).

†Geometric mean after logarithmic transformation (95% CI).

Serum t-IgE and s-IgE levels

A blood sample was processed at the time of diagnosis and before ASI. Serum t-IgE and s-IgE levels were determined by using the Unicap 100 with the fluoroimmunoassay technique (Phadia, Uppsala, Sweden), according to the manufacturer's instructions. Results for both serum t-IgE and s-IgE measurements were expressed in kilounits per liter and equilibrated against the World Health Organization standard for IgE: 1 kU for t-IgE and 1 kAU for s-IgE was equal to 2.4 ng/mL, respectively. Serum t-IgE levels were determined with a detection limit of 2 kU/L and an upper limit of 5000 kU/L. Serum s-IgE levels were determined with a detection limit of 0.35 kAU/L and an upper limit of 100 kAU/L. In all patients the s-IgE level was measured for the same allergens used in the skin prick tests (11 allergens).[15,16] All patients were monosensitized (had a positive skin prick test response for only 1 allergen and presented with s-IgE for the same allergen). For the calculation of the ratio, we used the following formula:

$$s-IgE/t-IgE \; ratio = \frac{s-IgE}{t-IgE} \times 100$$

Peripheral b-eos counts. Absolute peripheral b-eos counts were determined before ASI with a Technicon-H1 blood cell counter (Bayer, Leverkusen, Germany), with the normal range being 0.10 to 0.40 cells/10^{-3} μL.[17]

Statistical analysis

Statistical analysis was performed with the SYSTAT 10 software package (Systat Software, Inc, San Jose, Calif). Data were reported as the arithmetic

TABLE IV. Multivariate regression using the response to ASI as variable-dependent

Independent variables	*r*	*P* value
Age (y)	0.095	NS
Male/female sex	−0.150	NS
SCIT/SLIT	0.040	NS
Atopic family history	0.054	NS
Rhinitis/rhinitis plus asthma	0.101	NS
Onset of symptoms (y)	−0.075	.007
Pollens/HDM sensitization	0.105	NS
Serum s-IgE/t-IgE ratio	0.723	<.0001
Serum t-IgE level	−0.342	NS
Serum s-IgE level	0.316	.007
b-eos Counts	−0.429	<.0001
SPT response to allergens (mm)	0.009	NS

NS, not significant.

mean and 95% CI for the mean when the distribution of the data was normal. When normality was rejected, data were examined after logarithmic transformation and presented as the geometric mean and the 95% CI for the geometric mean. We used the Kolmogorov-Smirnov test to evaluate the normal distribution. Group comparison tests were performed with the 2-sided *t* test. Categorical variables between groups were compared by using the χ^2 test. An effective or ineffective response to ASI was assessed by examining the area under the ROC curves (c-statistic). Models with an ROC area of at least 0.80
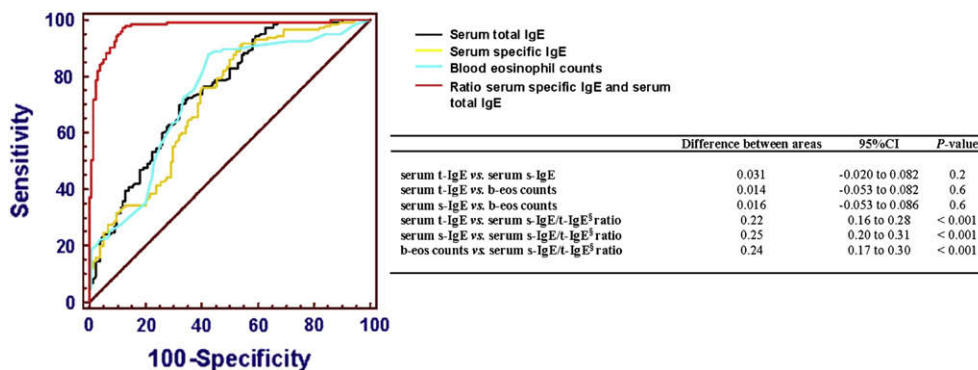
	Difference between areas	95%CI	P-value
serum t-IgE *vs.* serum s-IgE	0.031	-0.020 to 0.082	0.2
serum t-IgE *vs.* b-eos counts	0.014	-0.053 to 0.082	0.6
serum s-IgE *vs.* b-eos counts	0.016	-0.053 to 0.086	0.6
serum t-IgE *vs.* serum s-IgE/t-IgE§ ratio	0.22	0.16 to 0.28	< 0.001
serum s-IgE *vs.* serum s-IgE/t-IgE§ ratio	0.25	0.20 to 0.31	< 0.001
b-eos counts *vs.* serum s-IgE/t-IgE§ ratio	0.24	0.17 to 0.30	< 0.001

FIG 1. ROC curves obtained with serum t-IgE levels (decision point, ≤133 kU/L; sensitivity, 69.7%; and specificity, 67.9%), serum s-IgE levels (decision point, 9.5 kAU/L; sensitivity, 91.0%; specificity, 46.3%), b-eos counts (decision point, 0.43 cells × 10^{-3} μL; sensitivity, 87.6%; specificity, 57.5%), and serum s-IgE/serum t-IgE ratios (decision point, 16.2%; sensitivity, 97.2%; specificity, 88.1%) by plotting sensitivity in patients with an effective response to ASI versus 100-specificity in patients with an ineffective response to ASI.

were considered to have good predictive value.[18] For all analyses, a *P* value of less than .05 was considered statistically significant.

RESULTS

We examined a total of 120 male and 159 female subjects (mean age, 29.5 ± 8.1 years) meeting the criteria outlined above. One hundred fifty-two patients were affected by rhinitis alone, and 127 were affected by rhinitis plus asthma. Sixteen patients were monosensitized to grass, 135 to *P judaica*, 22 to *O europea*, and 106 to HDM. Two hundred three (72.8%) patients were treated with SLIT, and 76 (27.2%) were treated with SCIT. Response to ASI was considered effective in 145 (52.0%) patients: 4 sensitive to grass, 90 sensitive to *P judaica*, 3 sensitive to *O europea*, and 48 sensitive to HDM. ASI was considered clinically ineffective in 134 (48.0%) patients: 12 sensitive to grass, 45 sensitive to *P judaica*, 19 sensitive to *O europea*, and 58 sensitive to HDM.
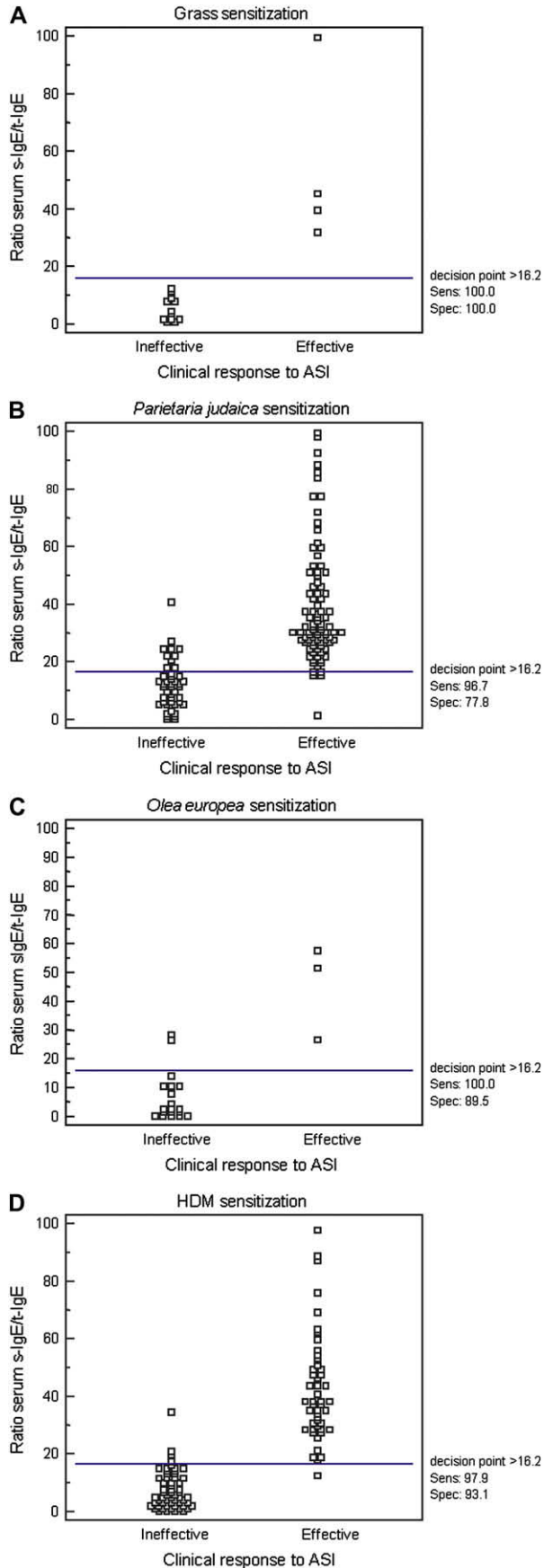
Patient characteristics are reported in Table I. Table II compares patients with an effective clinical response to ASI with those with an ineffective response, both as a group and according to the type of ASI (SLIT and SCIT). When comparing all the study patients, those who had an effective response to ASI differed significantly from those with an ineffective response in the following characteristics: sex (female subjects better than male subjects, *P* = .01), serum t-IgE levels (low levels better than high levels, *P* < .0001), serum s-IgE levels (high levels better than low levels, *P* < .0001), b-eos counts (low counts better than high counts, *P* < .0001), and serum s-IgE/t-IgE ratios (high ratios better than low ratios, *P* < .0001). Between patients with effective and ineffective clinical responses to SCIT, significant differences were found with regard to the following characteristics: sex (female subjects better than male subjects, *P* = .01), serum t-IgE levels (low levels better than high levels, *P* = .007), serum s-IgE levels (high levels better than low levels, *P* = .01), b-eos counts (low counts better than high counts, *P* < .0001), and serum s-IgE/t-IgE ratios (high ratios better than low ratios, *P* < .0001). Significant differences were found between patients with effective and ineffective clinical responses to SLIT with regard to serum t-IgE levels (low levels better than high levels, *P* < .0001), serum s-IgE levels (high levels better than low levels, *P* < .0001), b-eos counts (low counts better than high counts, *P* < .0001), and serum s-IgE/t-IgE ratios (high ratios better than

low ratios, *P* < .0001). We did not find a relationship among allergen dosing, total dose received, and clinical response because all the patients within each group received an equal dose.

Table III shows the characteristics of patients with rhinitis only and those with rhinitis and asthma symptoms. For patients with rhinitis only, significant differences were found between those with effective and ineffective clinical responses to ASI with regard to serum t-IgE levels (low levels better than high levels, *P* ≤ .0001), serum s-IgE levels (high levels better than low levels, *P* ≤ .0001), b-eos counts (low counts better than high counts, *P* < .0001), and serum s-IgE/t-IgE ratios (high ratios better than low ratios, *P* < .0001). For patients with rhinitis and asthma symptoms, significant differences were found between patients with effective and ineffective clinical responses to ASI with regard to serum t-IgE levels (low levels better than high levels, *P* ≤ .0001), serum s-IgE levels (high levels better than low levels, *P* ≤ .01), b-eos counts (low counts better than high counts, *P* < .0001), and serum s-IgE/t-IgE ratios (high ratios better than low ratios, *P* < .0001).

Serum t-IgE and s-IgE levels correlated with age (both showing), whereas the s-IgE/t-IgE ratio was not affected by age. Regression analysis showed that t-IgE levels (*r* = −0.13 [95% CI, −0.24 to −0.01], *P* = .02) and s-IgE levels (*r* = −0.17 [95% CI, −0.28 to −0.05], *P* = .004) were inversely related to age in years, whereas s-IgE/t-IgE ratios did not correlate with age (*P* = .3). In addition, using multivariate regression analysis, we found that response to ASI is independently related only to onset of symptoms, s-IgE/t-IgE ratios, s-IgE levels, and b-eos counts, again confirming that response to ASI is not related to age (Table IV).

Fig 1 shows the sensitivity and specificity obtained by calculating ROC curves for serum t-IgE levels, serum s-IgE levels, b-eos counts, and serum s-IgE/t-IgE ratios and the pairwise comparisons of the ROC curves. The area under the curve was 0.74 for serum t-IgE levels (95% CI, 0.69-0.79), 0.71 for serum s-IgE levels (95% CI, 0.65-0.76), 0.73 for b-eos counts (95% CI, 0.67-0.78), and 0.97 for serum s-IgE/t-IgE ratios (95% CI, 0.94-0.98). Significant differences were found between the s-IgE/t-IgE ratio and t-IgE levels alone (*P* < .001), s-IgE levels alone (*P* < .001), and b-eos counts (*P* < .001). No other significant differences were found.

FIG 2. Sensitivity *(Sens)* and specificity *(Spec)* calculated for each allergen used for ASI by using the ratio of serum s-IgE/serum t-IgE of 16.2% as the decision point. **A**, grass; **B**, *Parietaria judaica*; **C**, *Olea europa*; **D**, house dust mites (HDM).

Our ROC analysis of the serum s-IgE/t-IgE ratio showed that a ratio of greater than 16.2% had the best sensitivity (97.2%) and specificity (88.1%) to predict effective ASI, regardless of whether SCIT or SLIT was used. The sensitivity and specificity of the decision point for a serum s-IgE/t-IgE ratio of greater than 16.2%, as obtained from the ROC, was examined for each allergen used for immunotherapy. Results are as follows: sensitivity and specificity of 100% for grass; 96.7% and 77.8%, respectively, for *P judaica*; 100% and 89.5%, respectively, for *O europea*; and 97.9% and 93.1%, respectively, for HDM. This is shown in Fig 2. Fig 3 shows the means (± SD) of VAS scores with regard to rhinitis and asthma symptoms, respectively. Of note, rescue medication use decreased with improvement in symptoms, as reflected in the VAS. Likewise, medication use increased with worsened symptoms per the VAS (Tables E1-E3).

DISCUSSION

Our investigation shows that the serum s-IgE/t-IgE ratio significantly correlates with the clinical response to ASI administered through both the SCIT and SLIT routes. We considered the result of ASI to 4 aeroallergens epidemiologically very important in our geographic area (ie, grass, *P judaica*, *O europea*, and HDM) in a total of 279 patients.[14] Tests to predict the outcome of ASI would be of tremendous help in daily practice, but data about this aspect of immunotherapy have not been reported. Therefore we evaluated clinical, functional, and laboratory characteristics evaluated at the time of clinical diagnosis and analyzed the variables that were statistically different between the patients with effective and ineffective clinical responses to ASI after 4 years. These were serum t-IgE levels, serum s-IgE levels, b-eos counts, and serum s-IgE/t-IgE ratios. We compared these variables, considering the clinical response to ASI, apart from the route of administration. We found that for all the allergens considered, a serum s-IgE/t-IgE ratio of greater than 16.2% correlated with an effective clinical response to ASI with a sensitivity of 97.2% and a specificity of 88.1%. Our analyses demonstrated that the serum s-IgE/t-IgE ratio is superior to both serum t-IgE and s-IgE levels alone in predicting clinical response to ASI. This is based on only 1 measurement of s-IgE and t-IgE levels, which is a limitation of the study. Both samples were drawn at the time of diagnosis when patients were manifesting symptoms and therefore during a time when the patient was presumed to have a high clinical exposure. Measurements were obtained uniformly for all subjects at the time of diagnosis, generally during peak allergen exposure, thus reflecting a real-life situation comparable with the usual clinical practice for the management of such patients. Certainly, these considerations might be too speculative, and further studies with serial evaluations of IgE concentrations, including measurements during the season to look for the ablation of the seasonal increase in allergen s-IgE levels, are needed to confirm our results. Measuring allergen-specific IgG levels would also be useful because these are classically used to demonstrate response to ASI. It has been reported that the percentage of allergen s-IgE is approximately 25% of t-IgE.[19] Allergic inflammation is initiated by allergen molecules cross-linking their corresponding
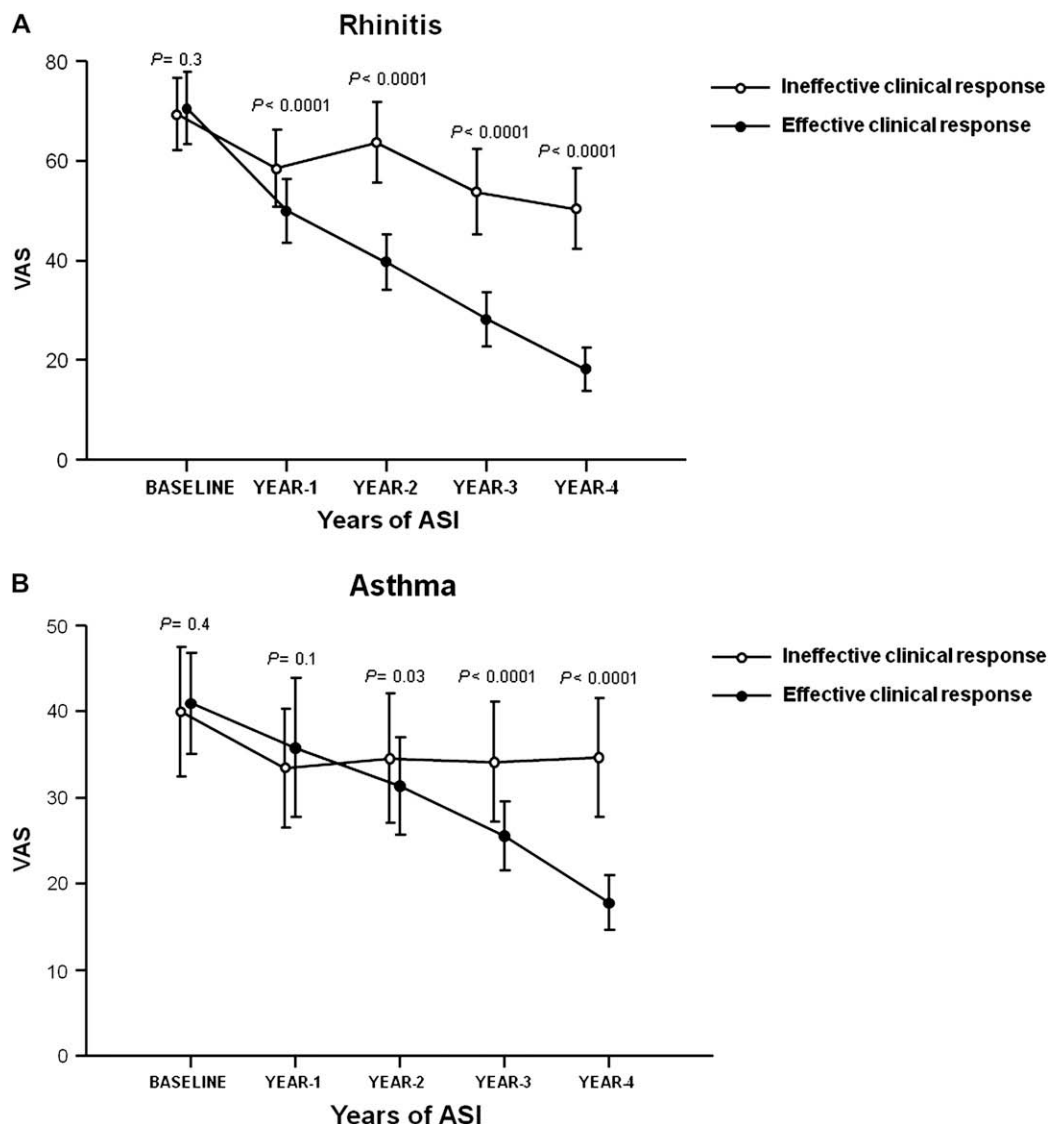
FIG 3. Graphic representation of means of VAS ($\pm$ SD) with regard to **A**, rhinitis and **B**, asthma symptoms, respectively.

receptor-bound s-IgE on the mast cell and basophil surface.[20,21] Although it is possible to quantify both serum t-IgE and s-IgE levels, serum t-IgE levels are not measured because they are not useful in the clinical diagnosis of allergy to common aeroallergens.[15] Thus a higher s-IgE/t-IgE ratio might reflect a higher level of allergen s-IgE on the mast cell and basophil surface, whereas this probability is lower when the serum s-IgE/t-IgE ratio is low.

For this reason, it might be helpful to measure the serum t-IgE level and calculate the serum s-IgE/t-IgE ratio. In fact, as shown by the area under the curve values of the ROC, this ratio is at least as accurate, if not better, than serum t-IgE and s-IgE levels alone at predicting clinical response to ASI. Calculating a decision point for the ratio that represents a 95% predicted probability for an effective clinical response for all 4 allergens might improve the sensitivity and specificity compared with both serum s-IgE and t-IgE levels alone. The predicted probability, calculated for each allergen studied, demonstrated that the cutoff level of greater than 16.2% showed both high specificity (between 77.8% for *P judaica*

and 100% for grass) and high sensitivity (between 96.7% for *P judaica* and 100% for grass and *O europea*).

The subjects in this study are monosensitized to the aeroallergens deemed most important in Italy.[14] We cannot completely exclude the possibility of sensitization to allergens not routinely tested, nor can we apply the findings to monosensitized patients. However, it is likely that the amount of s-IgE in the serum reflects the degree of environmental exposure to individual allergens. Thus it is conceivable that the higher the s-IgE/t-IgE ratio, the more clinically relevant the allergen is for a particular patient. Likewise, the more clinically relevant the allergens included in the ASI, the more successful ASI will be. A possible mechanism is that ASI generates allergen-specific regulatory T cells, and generating regulatory T cells specific to the patient's dominant and most relevant allergen might help generate sufficient regulatory T cells in the respiratory mucosa to suppress bystander IgE responses to other less relevant allergens, resulting in an overall decrease in mucosal allergic inflammation.[19]

ASI has proved efficacy for a variety of inhalant allergens. However, the treatment is of long duration. Because not all patients benefit from treatment, it would be useful to be able to have specific criteria to determine those patients who might best benefit from this therapy. We found that the serum s-IgE/t-IgE ratio is the best predictor of clinical response to allergen-specific immunotherapy. Although the s-IgE/t-IgE ratio has been already described in the medical literature, to the best of our knowledge, this is the first study that demonstrates a possible role of s-IgE/t-IgE ratio as a predictor of clinical response to allergen-specific immunotherapy. Our results are applicable only to patients monosensitized to these 4 allergens studied. In addition, the lack of precise data about pollen counts might be another limitation. However, there is no reason to think there was an important difference between years because the local registries for pollen counts did not reflect this. In addition, patients with no response to ASI required more rescue medication compared with those patients who responded, providing indirect evidence of adequate pollen counts.

Finally, the lack of a placebo is a limitation of this study. However, the aim of this study was not to perform a clinical trial that might be addressed in the future but to search for a potential explanation for why patients who have received ASI could have very different response rates and thus to search for possible predictors of such varied responses. Our study has a retrospective design and, consequently, has the common limitations of such types of studies. Nonetheless, we believe that these limitations do not significantly confound the main finding of our study. However, this needs to be further investigated, preferably through studies with a double-blind design, which is the gold standard for determining the efficacy of any therapy.

Clinical implications: The s-IgE/t-IgE ratio significantly correlated with the clinical response to ASI in monosensitized patients. This suggests that the s-IgE/t-IgE ratio can be used as a predictor of clinical response to immunotherapy.

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METHODS
Immunotherapy intervention

Dose administered of SCIT and SLIT. All the patients in this study tolerated the maximum dose indicated by the manufacturers' recommendations as follows. For SCIT, the maintenance dose was 0.8 mL, which corresponds to 1.6 μg of the major allergen of grass, 0.4 μg of the major allergen of *P judaica*, 9.6 μg of the major allergen of *O europea*, and 3.84 μg of the major allergens of *D pteronyssinus* and *D farinae*. For SLIT, the maintenance dose was 60 drops (1 drop = 25 μL) administered 3 times a week on alternate days, which corresponds to 28.2 μg of the major allergen of grass, 6.6 μg of the major allergen of *P judaica*, 169.2 μg of the major allergen of *O europea*, and 67.7 μg of the major allergens of *D pteronyssinus* and *D farinae*. These doses were given monthly. Immunotherapy with grass, *P judaica*, and *O europea* pollen was not interrupted during the spring, but the dose was halved.

The use of a perennial schedule with grass, *P judaica*, and *O europea* pollen was well tolerated in our patients, as shown by the absence of side effects associated with administration during the spring, the peak period of these pollens, both in Sicily and in Veneto.

We did not find a relationship between allergen dosing, total dose received, and clinical response because all the patients received an equal dose.

Assessment of symptoms and medication use. For clinical control, with regard to the 3 outdoor allergens, we performed clinical control and evaluation of the VAS for symptoms at the end of the season, specifically in June for patients allergic to grass and *O europea* and in September for patients allergic to *P judaica*. For patients allergic to *D pteronyssinus* and *D farinae*, we performed clinical control and evaluation of the VAS for symptoms at the end of February because they are typically increased in autumn, after vacations, and during winter.

In Sicily, however, there is not a well-defined season for grass and *P judaica*. Thus the use of medication and, perhaps, symptom scoring might be outside the pollen season and therefore might be relevant to the assessment of the efficacy of immunotherapy.

Evaluation of clinical response. The clinical response to ASI was evaluated with separate VASs for rhinitis and asthma. For rhinitis, the patients assessed their total nasal symptom scores (sneezing, rhinorrhea, nasal congestion, and nasal pruritis) yearly for 4 years with the VAS. Subjects were instructed that 0 indicated "nasal symptoms not at all bothersome" and that 100 indicated "nasal symptoms extremely bothersome." For asthma, the patients did the same, marking the VAS for all asthma symptoms yearly for 4 years (shortness of breath, chest tightness, cough, and wheezing). Each VAS was 100 mm horizontal with 0 and 100 on the left and right ends, respectively. Subjects were instructed that 0 indicated "no complaints of respiratory sensation, such as shortness of breath, chest tightness, and breathlessness" and 100 indicated "the worst complaints of respiratory symptoms imaginable." The distance between 0 and the marks the subjects made on the scale was measured with a Digimatic caliper (Mitutoyo, Kawasaki, Japan).

Rescue medication included an H_1-antagonist for relief of rhinitis symptoms (loratadine [Claritin], 10 mg; Schering-Plough, Segrate, Italy) and a short-acting β_2-agonist for relief of asthma symptoms (salbutamol [Ventolin], 100 μg per actuation of a metered-dose inhaler; GlaxoSmithKline, Naples, Italy). We asked patients to indicate the number of packages of loratadine tablets (20 tablets per package) taken in each year and the number of salbutamol inhalers used in each year. The scale of rescue medication was between 0 and 3. The subjects were instructed that 0 indicated no rescue medication; 1 indicated a maximum of 1 package of loratadine (≤20 tablets per year; see Table E1) and 1 salbutamol inhaler (≤20 mg/y); 2 indicated a maximum of 3 packages of loratadine (≤60 tablets per year) and a maximum of 4 salbutamol inhalers (≤ 80mg/y); and 3 indicated 4 or more packages of loratadine and 5 or more salbutamol inhalers (Table E2).

Comparison between SCIT and SLIT in patients with effective clinical responses to ASI. The comparison between SCIT and SLIT in patients with an effective clinical response to ASI is shown in Table E3. Significant differences were noted only for atopic family history ($P < .0001$) and serum s-IgE levels ($P = .02$).

TABLE E1. Short-acting β_2-agonist used as rescue medication for asthmatic symptoms

	No. of patients			
	Ineffective clinical response (n = 54)		Effective clinical response (n = 73)	
Rescue medication	Before ASI	After ASI	Before ASI	After ASI
0 (no rescue medication)	0	0	0	68
1 (mild use)	10	13	9	5
2 (moderate use)	22	30	35	0
3 (major use)	22	11	29	0

TABLE E2. H$_1$-antagonist used as rescue medication for symptoms of rhinitis

	No. of patients			
	Ineffective clinical response (n = 80)		Effective clinical response (n = 72)	
Rescue medication	Before ASI	After ASI	Before ASI	After ASI
0 (no rescue medication)	0	0	0	0
1 (mild use)	0	0	0	70
2 (moderate use)	22	30	21	2
3 (major use)	58	50	51	0

TABLE E3. Patients with effective clinical responses to ASI: Comparison between SCIT and SLIT

	SCIT	SLIT	*P* value
Patients, no. (%)	42	103	
Age (y)*	29.8 (26.8-32.7)	30.5 (28.9-32.1)	.6
Male/female sex	11/31	41/62	.1
Male subjects (%)	26.2	39.8	
Female subjects (%)	73.8	60.2	
Atopic family history (yes/no)	36/6	28/75	<.0001
Positive family history group (%)	85.7	27.2	
Negative family history group (%)	14.3	72.8	
Passive smoking (yes/no)	16/26	49/54	.3
Positive smoking group (%)	38.1	47.6	
Negative smoking group (%)	61.9	52.4	
Rhinitis/rhinitis plus asthma	22/20	50/53	.8
Rhinitis group (%)	52.4	48.5	
Rhinitis-asthma group (%)	47.6	51.5	
Onset of symptoms (y)*	7.1 (5.4-8.7)	6.5 (5.5-7.5)	.5
Pollens/HDM sensitization	24/18	73/30	.6
Pollens group (%)	57.1	70.9	
HDM group (%)	42.9	29.1	
Grass, no. (%)	1 (2.3)	3 (2.9)	
P judaica, no. (%)	23 (54.8)	67 (65.0)	
O europea, no. (%)	0	3 (2.9)	
HDM, no. (%)	18 (42.9)	30 (29.1)	
SPT response to allergen (mm)†	5.4 (5.2-5.6)	5.4 (5.2-5.6)	.8
Serum t-IgE level (kU/L)†	105.2 (80.8-137.1)	76.9 (64.4-91.8)	.09
Serum s-IgE level (kAU/L)†	38.5 (28.5-51.9)	27.7 (23.1-33.3)	.02
b-eos Counts (cells $\times$ 10^{-3} μL)†	0.30 (0.27-0.36	0.31 (0.29-0.33)	.5
Serum s-IgE/t-IgE* ratio	36.6 (29.6-45.2)	36.1 (33.1-39.3)	.4
Patient no. (%) with s-IgE/t-IgE ratio >16.2%	41 (97.6)	100 (97.1)	.8
Patient no. (%) with s-IgE/t-IgE ratio ≤16.2%	1 (2.4)	3 (2.9)	

SPT, Skin prick test.
*Mean (95% CI).
†Geometric mean after logarithmic transformation (95% CI).