Pollen Allergy Screening with Allergen-Specific and Total Immunoglobulin E Titers

Hidenori Yokoi, MD, PhD[1] [ID], Yuma Matsumoto, MD, PhD[1],
Michitsugu Kawada, MD, PhD[1], Hiroyuki Sakurai, MD, PhD[2],
and Koichiro Saito, MD, PhD[1]

Abstract

Background: Allergic rhinitis is a typical type I hypersensitivity reaction, commonly caused by inhalant allergens. Accurate identification of the causative antigen is important for rapid diagnosis and treatment initiation.

Objective: This study examined the efficiency of serum-based allergen-specific immunoglobulin E and total immunoglobulin E antibody titers in screening for pollen allergy. We also examined the effect of cross-reactive carbohydrate determinants on specific immunoglobulin E titers in screening for pollen allergy, one of the causes of false positivity in specific immunoglobulin E measurements.

Methods: A questionnaire was used to evaluate the symptoms of pollinosis among participants who underwent a medical examination. One hundred and thirty-two participants reported pollen allergy symptoms and 127 reported an absence of symptoms. Specific immunoglobulin E levels were measured using the AlaSTAT 3g Allergy method. Seventeen components, including four types of cross-reactive carbohydrate determinant-specific immunoglobulin E antibodies, were measured and evaluated comparatively.

Results: The sensitivity and specificity of the tests in predicting the presence or absence of pollen allergy were analyzed. The values of the areas under the curves for immunoglobulin E antibody levels against cedar, cypress, orchard grass, and ragweed pollen were 0.87, 0.82, 0.63, and 0.56, respectively. A cross-reactive carbohydrate determinant-related false-positive effect on the pollen specific immunoglobulin E titer was noted in pollen screening.

Conclusion: Cedar pollen-specific immunoglobulin E titers showed sufficient accuracy for use in pollen allergy screening. The study of cross-reactive carbohydrate determinants suggested that subjects who tested positive for pollen often had false-positive results due to the impact of cross-reactive carbohydrate determinants.

Keywords

pollen allergy, allergen, immunoglobulin E, carbohydrates, surveys and questionnaires, rhinitis/seasonal

Introduction

It is well known that the prevalence of allergic rhinitis is rising worldwide.[1] This allergic disease imposes a significant financial burden on patients and their families, and thus, accurate diagnosis is important before starting treatment.[1,2] Allergic rhinitis is a typical type I hypersensitivity reaction and is commonly caused by inhalant allergens, including plant pollen, mites, and house dust.[3] Japanese cedar pollinosis, a specific form of allergic rhinitis, presents with symptoms of varying severity.[4] Allergic rhinitis can be treated with the following: 1) drug therapies: antihistamines, leukotriene receptor antagonists, and intranasal corticosteroid spray; 2) surgical interventions; and 3) allergen immunotherapy.[1,5–7] Among them, allergen avoidance is the base line to prevent allergic rhinitis.[1,8] However, it is difficult to completely avoid inhaled antigens; therefore, it is crucial to accurately identify

[1]Department of Otolaryngology, Head and Neck Surgery, Kyorin University School of Medicine, Tokyo Japan
[2]Department of Pharmacology, Kyorin University School of Medicine, Tokyo Japan

Corresponding Author:
Hidenori Yokoi, MD, PhD, Department of Otorhinolaryngology-Head and Neck Surgery, Kyorin University School of Medicine, 6-20-2 Shinkawa, Mitaka, Tokyo 181-8611 Japan.
E-mail: h-yokoi@ks.kyorin-u.ac.jp

the causative antigen for rapid diagnosis and prompt initiation of treatment of allergen immunotherapy.

The causative antigen is generally identified using allergen-specific immunoglobulin E (sIgE) antibody tests, skin allergy tests (eg, intracutaneous and scratch tests), and allergen-induced tests. Serum-sIgE tests with fluorescence-enzyme immunoassay, chemiluminescent enzyme immunoassay, and enzyme immunoassay are often favorably chosen in clinical settings due to the availability of tests for different allergen-sIgE antibodies with relatively high specificity.[9]

The presence of allergen-sIgE antibodies is indicative of sensitization to allergens. However, frequently in routine clinical settings, the presence of allergic sensitization does not coincide with symptoms. This study aimed to evaluate the effectiveness of serum-based allergen-sIgE antibody measurements for the screening of pollen allergy. Furthermore, although there are reports on the possibility of using biomarkers for some atopic diseases,[10] the significance of total IgE (tIgE) measurements for pollen allergy remains unknown. Therefore, the significance of tIgE measurements was also assessed in this study. Concomitantly, we also examined the influence of cross-reactive carbohydrate determinant (CCD) antigens, which are cross-reactive protein-linked carbohydrate moieties that have been reported to confer false positivity during allergen-sIgE antibody testing.[11–13]

Materials and Methods

Study Participants

This study recruited employees from a private company who underwent health screening in September 2015, wherein allergy testing was a part of the general health check-up. We evaluated 259 adults (189 men and 70 women; mean age = 40.3 [19-68] years). A self-reported questionnaire was used to evaluate the symptoms of pollinosis; the main question asked was "Are you suffering from pollinosis?" and the respondents answered in five different ways: I. Yes. I was diagnosed with pollinosis; II. Yes. Though I

was not diagnosed, I have symptoms; III. I do not know; IV. No. I have had symptoms in the past but not in recent times; V. No. I have no symptoms. Respondents who answered I or II were also asked about the three symptoms of pollinosis and their severity, and all of them had at least one or more symptoms, with a severity of $\geq 1+$ [14] (Table 1). Therefore, those who answered I or II were included in the pollen allergy group (group A), and those who answered IV or V were included in the pollen allergy-free group (group B). Respondents who answered III were removed from this study. Further, participants underwent allergen-sIgE antibody and tIgE measurements as part of their health examination. The study protocol was approved by the Institutional Review Board of Kyorin University (No. 427). Written informed consent was obtained from the employees for their participation and use of data. This study was conducted in accordance with the principles of the Declaration of Helsinki.

Measurement of Allergen-sIgE Antibody Titers and tIgE in Blood serum

We evaluated the roles of sIgE and tIgE levels in the diagnostic screening of pollinosis. Concomitantly, we also evaluated CCD antigens. Serum samples were collected from the venous blood samples of the subjects and preserved at −20°C; until further use. IMMULITE® 2000 Systems 3g Allergy™ (IML 3g Allergy; Siemens Healthcare GmbH, Erlangen, Germany), a chemiluminescent enzyme immunoassay, was used for measuring the titers of sIgE against the following allergens: pollen of Japanese cedar, Japanese cypress, orchard grass, and common ragweed; and MUXF, bromelain, horseradish peroxidase (HRP), and ascorbate oxidase (ASOD) as CCD allergens. Allergen-sIgE titers were defined as follows: negative, < 0.10 IU_A/mL (Class 0); slightly positive, 0.10–0.34 IU_A/mL (Class 0 +); low positive, 0.35–0.69 IU_A/mL (Class 1); moderate positive, 0.70–3.49 IU_A/mL (Class 2); high positive, 3.5–17.49 IU_A/mL (Class 3), 17.5.–52.49 IU_A/mL (Class 4); and very high positive, 52.5–99.99 IU_A/mL (Class 5), ≥ 100 IU_A/mL (Class 6).

Table 1. Symptoms and Severity of Allergic Rhinitis.

Types	Severity				
	++++	+++	++	+	-
Paroxysmal sneezing (average number of episodes of paroxysmal sneezing in a day)	≥21 times	20-11 times	10-6 times	5-1 times	Below +
Rhinorrhea sneezing (average number of episodes of nose blowing in a day)	≥21 times	20-11 times	10-6 times	5-1 times	Below +
Nasal blockage	Completely obstructed all day	Severe nasal blockage causing prolonged oral breathing in a day	Severe nasal blockage causing occasional oral breathing in a day	Nasal blockage without oral breathing	Below +

Furthermore, the tIgE value (using the sandwich enzyme-linked immunosorbent assay method) was also measured for each sample.

Statistical Analysis

Statistical analysis was performed using Excel (Microsoft Corp, Redmond, WA, USA) and SAS v9.4 (SAS Institute, Cary, NC, USA) software. Excel analysis tools (t-test and chi-square test) were used to assess the significance of the differences in sIgE levels between the two groups and the relationship between false-positive results in sIgE tests and CCDs in the without-symptom group. SAS v9.4 was used to run multivariate logistic regression, visualize relationships by cubic spline, and perform receiver operating characteristic (ROC) analysis, which were the main methods used to predict symptoms. The cut-point was computed with the Youden Index.

Results

Positivity Rates of Allergen-SIgE in the two Groups

There were 132 participants in group A and 127 in group B. In total, 88 participants were excluded from this study. The allergen-specific serum IgE positivity rates in group A compared to that in group B were as follows: 97% versus 56% (p < 0.01) for Japanese cedar, 91% versus 35% (p < 0.01) for Japanese cypress, 39% versus 15% (p < 0.01) for orchard grass, and 14% versus 3% for common ragweed (p = 0.10), respectively. Significant differences were noted in the allergen-sIgE positivity rates for pollen antigens of cedar, cypress, and orchard grass between the groups.

Pollen-SIgE Class Distribution of Japanese Cedar in the Study Groups

The allergen-sIgE positivity rates in participants with and without symptoms were as follows: class 0 (9% and 65%), class 0 + (11% and 6%), class 1 (11% and 8%), class 2 (30% and 13%), class 3 (33% and 7%), class 4 (6% and 2%), class 5 (0% and 0%), and class 6 (0% and 0%), respectively. In classes 0–1, 30% of participants had symptoms, while 79% did not. In classes 2–6, 70% of participants had symptoms, while 21% did not. The symptom positivity rate peaked in classes 2–3 (Figure 1).

Sensitivity and Specificity of Japanese Cedar-SIgE Antibody Values in Predicting the Presence or Absence of Pollen Allergy

A high area under the curve (AUC) value for the Japanese cedar-sIgE antibody was obtained (0.87). The cut-off value, which showed a high sensitivity of 87.13% and specificity of 76.14%, was 2.54 IU_A/mL and was included in class 2. Figure 2 shows the ROC curve.

Pollen-SIgE Class Distribution of Japanese Cypress in the Study Groups

The allergen-sIgE positivity rates in participants with and without symptoms were class 0 (3%, 44%), class 0 + (2%, 7%), class 1 (2%, 7%), class 2 (16%, 20%), class 3 (33%, 16%), class 4 (36%, 4%), class 5 (6%, 0%), and class 6 (2%, 2%), respectively. In classes 0–1, 6% of
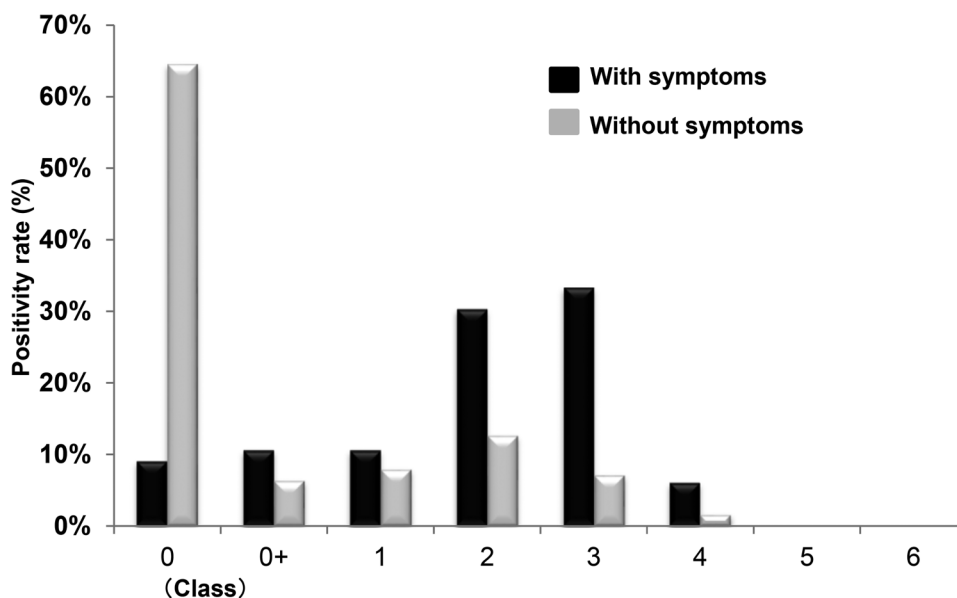

Figure 1. Class classification (Japanese cedar). The x-axis represents the class based on the presence or absence of hay fever symptoms and the y-axis represents the positivity rate of Japanese cedar-specific anti-IgE.

participants had symptoms and 58% had no symptoms. In classes 2–6, 94% of participants had symptoms and 42%

had no symptoms. The symptoms-positivity rate peaked in classes 3–4 (Figure 3).
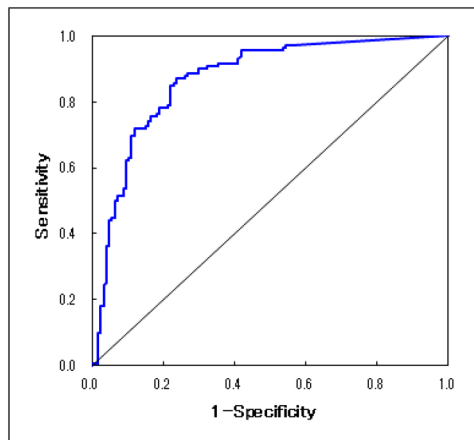
ROC AUC	: 0.87
Sensitivity	: 87.13 %
Specificity	: 76.14 %
Cut-off value	: 2.54 IUA/ml (Class 2: 0.70~3.45 IUA/ml)

Figure 2. Japanese cedar: ROC curve. The ROC curve of the sensitivity/specificity results for Japanese cedar-specific anti-IgE and hay fever symptoms, where the x-axis represents specificity, and the y-axis, sensitivity. ROC AUC: receiver operating characteristic-area under the curve.

Sensitivity and Specificity of Japanese Cypress-SIgE Antibody Values in Predicting the Presence or Absence of Pollen Allergy

AUC analysis for the presence or absence of pollen allergy from Japanese cypress-sIgE antibodies showed a value of 0.82. The cut-off value, which showed a sensitivity of 90.20% and a specificity of 67.74%, was 0.13 IU_A/mL and was included in class 0+. Figure 4 shows the results of the ROC analysis.

Sensitivity and Specificity of Orchard Grass-SIgE Antibody Values in Predicting the Presence or Absence of Pollen Allergy

AUC analysis showed a value of 0.63. The cut-off value, which showed a sensitivity of 39.41% and a specificity of 85.04%, was 0.10 IU_A/mL and was included in class 0+ (ROC curve data not shown).

Sensitivity and Specificity of Common Ragweed-sIgE Antibody Values in Predicting the Presence or Absence of Pollen Allergy

AUC analysis showed a value of 0.56. The cut-off value, which showed a sensitivity of 13.63% and a high specificity of 97.64%, was 0.11 IU_A/mL and was included in class 0+ (ROC curve data not shown).
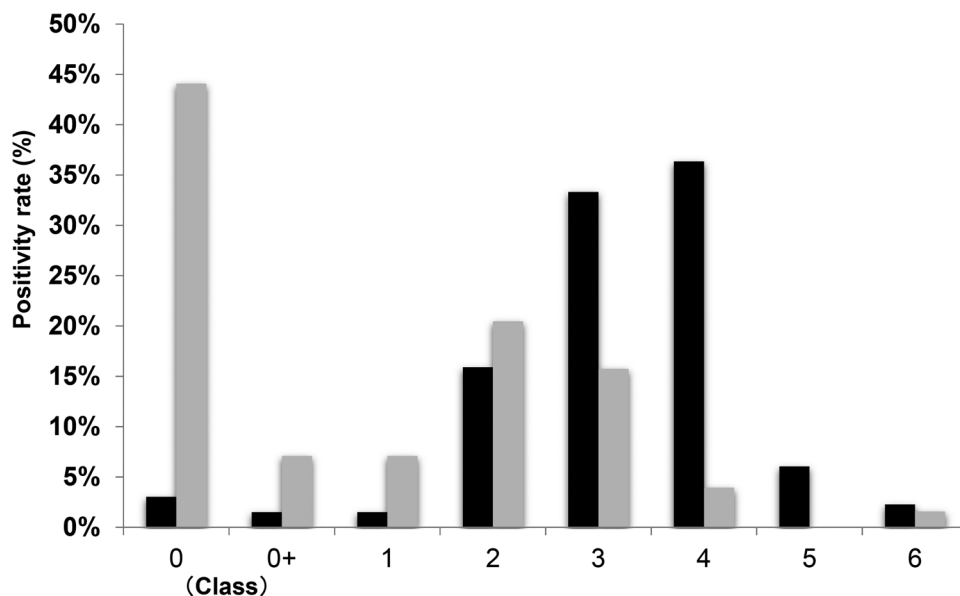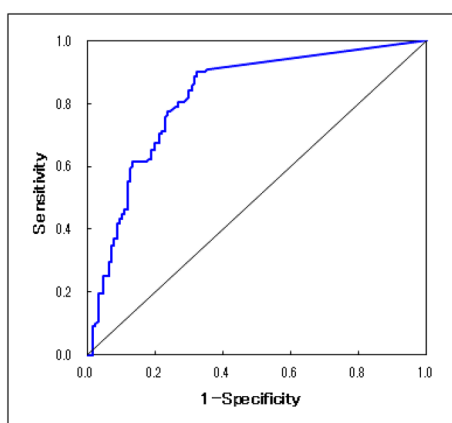

Figure 3. Class classification (Japanese cypress). The x-axis represents the class based on the presence or absence of hay fever symptoms and the y-axis represents the positivity rate of Japanese cypress-specific anti-IgE.

ROC AUC	: 0.82
Sensitivity	: 90.20 %
Specificity	: 67.74 %
Cut-off value	: 0.13 IUA/ml (Class 0+: 0.10~0.34 IUA/ml)

Figure 4. Japanese cypress: ROC curve. The ROC curve of the sensitivity/specificity results for Japanese cypress-specific anti-IgE and hay fever symptoms, where the x-axis represents specificity, and the y-axis, sensitivity. ROC AUC: receiver operating characteristic-area under the curve.

The Related Antigen Positivity Rate of CCD-Positive/Negative Groups

Of all the 259 cases examined, 43 cases were positive for any of the four CCDs and 216 cases were negative for CCD. In the CCD-positive group, 30 had pollen allergy symptoms, and the positivity rate for each antigen was as follows: Japanese cedar, 97%; Japanese cypress, 97%; orchard grass, 57%; and ragweed, 27%. The positivity rate of each antigen in the 13 CCD-positive cases without pollen symptoms was as follows: Japanese cedar, 85%; Japanese cypress, 77%; orchard grass, 62%; and ragweed, 31%. In the CCD-negative group, 102 cases with pollen allergy symptoms showed positive rates for the following antigens: Japanese cedar, 97%; Japanese cypress, 89%; orchard grass, 34%; and ragweed, 11%. The antigen positivity rates in the 114 cases without pollen symptoms were as follows: Japanese cedar, 53%; Japanese cypress, 31%; orchard grass, 10%; and ragweed, 0% (Table 2).

Relationship Between False-Positives for SIgE Tests and CCD

Relationship Between Japanese Cedar-sIgE Antibody and CCD. In the without-symptom group (127 cases), 71 cases were Japanese cedar-sIgE-positive (false-positive rate was 5%); of the 71 false-positive cases, 11 were CCD-positive. In the

Table 2. Antigen Positivity Rates in the CCD-Positive and CCD-Negative Groups. CCD: Cross-Reactive Carbohydrate Determinant.

	All subjects (n = 259)		
	With symptom (n = 132) Positivity rate (%)	Without symptom (n = 127) Positivity rate (%)	P-value
Japanese cedar	97%	56%	P < 0.01
Japanese cypress	91%	35%	P < 0.01
Orchard grass	39%	15%	P < 0.01
Common ragweed	14%	3%	Not significant
	CCD-negative (n = 216)		
	With symptom (n = 102) Positivity rate (%)	**Without symptom (n = 114) Positivity rate (%)**	P-value
Japanese cedar	97%	53%	P < 0.01
Japanese cypress	89%	31%	P < 0.01
Orchard grass	34%	10%	P < 0.01
Common ragweed	11%	0%	P < 0.05
	CCD-positive (n = 43)		
	With symptom (n = 30) Positivity rate (%)	**Without symptom (n = 13) Positivity rate (%)**	P-value
Japanese cedar	97%	85%	Not significant
Japanese cypress	97%	77%	Not significant
Orchard grass	57%	62%	Not significant
Common ragweed	27%	31%	Not significant

Table 3. Observed and Expected Frequencies of Pollen-SIgE and CCD Positivity in the Without Symptom Group (n = 127). CCD: Cross-Reactive Carbohydrate Determinant; SIgE: Specific IgE.

		Japanese cedar sIgE			
		Positive	Negative	Total	P < 0.05
CCD	Positive	11 (7.27)	2 (5.73)	13	
	Negative	60 (63.73)	54 (50.27)	114	
	Total	71	56	127	
		Japanese cypress sIgE			
		Positive	**Negative**	**Total**	**P < 0.05**
CCD	Positive	10 (4.61)	3 (8.39)	13	
	Negative	35 (40.39)	79 (73.61)	114	
	Total	45	82	127	
		Orchard grass sIgE			
		Positive	**Negative**	**Total**	**P < 0.05**
CCD	Positive	8 (1.94)	5 (11.06)	13	
	Negative	11 (17.06)	103 (96.94)	114	
	Total	19	108	127	
		Common ragweed sIgE			
		Positive	**Negative**	**Total**	**P < 0.05**
CCD	Positive	4 (0.41)	9 (12.59)	13	
	Negative	0 (3.59)	114 (110.41)	114	
	Total	4	123	127	

() Expected frequency.

CCD-positive group, 11 cases were Japanese cedar-sIgE-positive and two were Japanese cedar-sIgE- negative (Table 3).

Relationship Between Japanese Cypress-sIgE Antibody and CCD. In the without-symptom group (127 cases), 45 cases were Japanese cypress-sIgE-positive (35% false-positive rate); of the 45 false-positive cases, 10 were CCD-positive. In the CCD-positive group, 10 cases were Japanese cypress-sIgE-positive and 3 were Japanese cypress-sIgE-negative (Table 3).

Relationship Between Orchard Grass-sIgE Antibody and CCD. In the without-symptom group (127 cases), 19 cases were orchard grass-sIgE-positive (15% false-positive rate); of the 19 false-positive cases, 8 were CCD-positive. In the CCD-positive group, 8 were orchard grass-sIgE-positive and 5 were orchard grass-sIgE-negative (Table 3).

Relationship Between Common Ragweed-sIgE Antibody and CCD. In the without-symptom group (127 cases), 4 cases were common ragweed-sIgE positive (3% false-positive rate). Of the 4 false-positive cases, all 4 were CCD-positive. However, 9 cases were common ragweed-sIgE-negative and CCD-positive (Table 3).

Tests of significance for 2×2 contingency tables were conducted to determine whether the pollen-sIgE values of Japanese cedar, Japanese cypress, orchard grass, and common ragweed in the without symptom group (n = 127) differed between the CCD-positive and negative cases. The
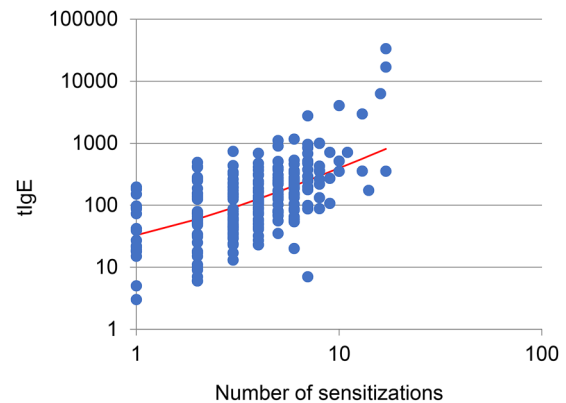

Figure 5. Relationship between tIgE levels and the number of sensitizations. The graph of Pearson's correlation coefficients ln (tIgE value + 1) and ln (number of sensitizations + 1), showing a moderate positive correlation between tIgE levels and the number of sensitizations (r = 0.67). tIgE: total immunoglobulin E.

test, with a significance level of 5%, showed a relationship with a sIgE value (Table 3).

Relationship Between the tIgE Values and Number of Sensitizations

To examine the role of tIgE levels in the diagnostic screening of pollen allergy, the correlation between the tIgE values and number of sensitizations was analyzed. Pearson correlation

coefficients ln (tIgE + 1) and ln (sensitization number + 1) were used to assess the accuracy and precision of the analysis. A moderately positive correlation was observed with r = 0.67 (Figure 5).
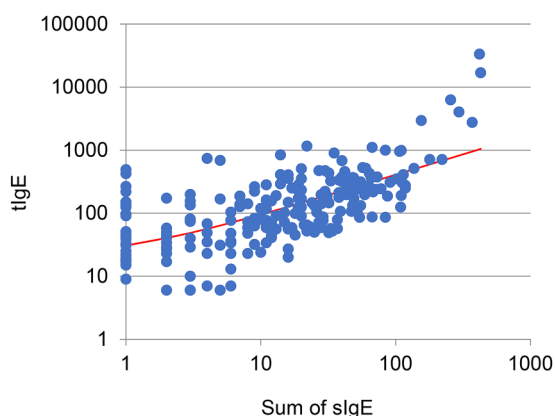
Relationship between tIgE and sIgE Values

To further examine the effect of tIgE levels on the diagnostic screening of pollinosis, the correlation between tIgE and sIgE values was analyzed. Pearson correlation coefficients (ln (tIgEs + 1) and ln (sum of sIgE + 1)) were used to assess the accuracy and precision of the analysis. A moderately positive correlation was observed with r = 0.70 (Figure 6).

Figure 6. Relationship between tIgE levels and sIgE. The graph of Pearson's correlation coefficients ln (tIgE value + 1) and ln (sIgE sum total + 1), showing a moderate positive correlation between tIgE levels and sIgE (r = 0.70). tIgE: total immunoglobulin E; sIgE: specific immunoglobulin E.

Sensitivity and Specificity of tIgE Antibody Values in Predicting the Presence or Absence of Pollen Allergy （Symptom Positive: 132 Cases, Symptom Negative: 124 Cases）
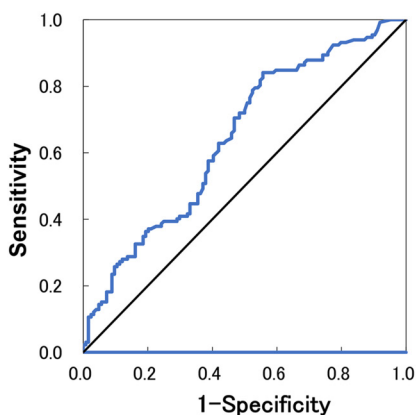
To determine the sensitivity/specificity of tIgE levels in the diagnostic screening for pollinosis, ROC curve analysis was performed. The AUC was 0.64 (confidence interval = 95% [0.58, 0.71]). Figure 7 shows the ROC curve for participants with/without symptoms compared to tIgE levels.

Discussion

So far, the usefulness of allergen-sIgE, tIgE, and CCD measurements in the context of pollen allergy screening during health examinations has not been explored. Our results showed that cedar pollen-sIgE values provided sufficient performance for use in pollen allergy screening. Simultaneous measurements of tIgE levels had limited ability to differentiate whether or not a pollen allergy was present. Further, it was difficult to set a statistical cut-off value for tIgE, casting doubt on its usefulness in screening for pollen allergies.

The World Health Organization recommends a skin test, especially using the prick method, for early-stage antigen identification to diagnose type I allergy, which involves IgE.[15] Although sIgE testing is more expensive, it involves a simple test for examining the allergen-sIgE antibody titer in serum; the amount of antibody can be objectively quantified, there is no risk of anaphylaxis, and there is no restriction on drug use before the test. It has high sensitivity and specificity, even when compared to diagnosis by both clinical symptoms and prick test.[16]

Presently, the latest, third-generation system, IMMULITE (IML) 3gAllergy, is used to test the allergen-sIgE antibody

ROC AUC	: 0.64
Sensitivity	: 51.9 %
Specificity	: 59.7 %
Cut-off value	: 87.0 IU/ml

Figure 7. Total IgE of participants: ROC curve. The ROC curve of the sensitivity/specificity results for symptom-positive participants (n = 132) and symptom-negative participants (n = 124) and their tIgE levels, where the x-axis represents specificity, and the y-axis represents sensitivity. ROC AUC: receiver operating characteristic-area under the curve.

titer using serum. AlaSTAT 3g is a new test that measures sIgE antibodies, and the measurement is based on chemiluminescent enzyme immunoassay (CLEIA)—a 2-step sandwich using a liquid-phase allergen and beads as the solid phase. The use of CLEIA enables accurate, wide-range measurement using only 0.10–500 IU$_A$/mL of reagents. In terms of classification, a value of 0.35 UA/mL is determined to be negative with the second-generation ImmunoCAP® technology (CAP), the testing method that has been most widely used so far. However, in our study with AlaSTAT 3g, a value of <0.10 IU$_A$/mL was denoted as negative, and a value of 0.10–0.35 IU$_A$/mL was denoted as 0 + (weakly positive). In light of a recent report [17] that compared IML with the skin test, the area under the ROC curve was the same, at least for IML and CAP in terms of the nine allergens that were tested, and the wider testing range of IML was partially reflected. However, in this study, IML 3g Allergy was used.

When the sIgE-positive cases were separated into the pollen allergy and pollen allergy-free groups, the cedar allergen-sIgE antibody positive rate and the cypress allergen-sIgE antibody positive rate in the pollen allergy group were over 90%. Pollen allergy patients who tested negative for cedar- or cypress allergen-sIgE antibodies could be deemed to not have developed cedar or cypress pollen allergy. Interestingly, the questionnaire screening showed a statistically significant difference only when the sIgE-positive rate was divided according to the presence or absence of symptoms for cedar, cypress, and orchard grass pollen allergies. There was also a considerable difference for ragweed, but it was not significant. The sIgE-positive rates for orchard grass and ragweed in these groups were low, at 38% and 14%, respectively, suggesting that in terms of pollen allergy in Japan, the prevalence and symptoms, or the absence thereof, are closely related to cedar and cypress pollens.

Further, regarding the results from studying the sIgE class distribution in the pollen allergy and pollen allergy-free groups, a ROC curve was created to investigate the sensitivity/specificity of sIgE antibody levels for predicting pollen allergy. The degree of association between the allergen-sIgE values and the presence or absence of symptoms was in the following order: cedar>cypress>orchard grass>ragweed. Screening tests using serum suggested that it was most useful in cases of Japanese cedar pollen.

CCDs are carbohydrate structures of glycoprotein allergens in pollen that resemble parts of carbohydrate structures of glycoprotein allergens in food. Thus, even patients who reacted to pollen allergens and were positive for allergen-sIgE antibodies for a food allergen have reportedly not had symptoms on an oral challenge with that food. These carbohydrates shared by pollens and foods are called CCDs, and allergen-sIgE antibodies to CCDs reportedly exist.[11,12] The involvement of CCD-sIgE antibodies in pollen allergy testing has been previously reported.[13] To

confirm the existence of CCDs and to analyze the ability of CCDs to cause false positives, which may indicate cross-reactivity of allergen-sIgE antibodies with antigen-specific CCDs, inhibition tests were performed. In these tests, CCD extracts (ten-fold serial dilutions of the inhibitor solution) were prepared as inhibitor solutions. The dilutions were individually dispensed into patient samples and into IgE-negative serum controls (negative controls). The results revealed that a CCD was marginally present in some cases, suggesting that 28.6% of participants had false-positive results.[13]

In the present study, the positive rates for orchard grass- and ragweed-sIgE antibodies were clearly high in the CCD-positive group, and there was a remarkable difference, in particular, in the pollen allergy-free group. This suggests that CCD-related antigens are involved in these sIgE antibody measurements (Table 2). In fact, statistical studies showed that the CCD-negative group had a significant difference between the with-symptom and without-symptom groups for each type of pollen. However, the false-positive results due to CCDs in the CCD-positive group suggested that there was no significant difference between the with-symptom and without- symptom groups for each pollen type (Table 2).

Furthermore, as a result of analyzing the relationship between false-positives for sIgE tests and CCDs in the without symptom group, a significant difference level of 5% was found in each of the four pollen types (P < 0.05) (Table 3).

In the quantification of IgE levels using serum for type I allergic diseases, the usefulness of tIgE levels was also examined in the screening test for pollen allergy. There have been several prior studies on the significance of tIgE levels in type I allergic diseases. In the assessment of tIgE, there is reportedly a strong correlation between tIgE levels and atopic sensitization.[18] Chung et al [19] suggested that patients with low tIgE levels may not need sIgE testing, but in those with high tIgE levels, specific sensitization of a patient with allergic rhinitis can be confirmed with sIgE testing. However, it cannot yet be said that the relationship between IgE levels and pollen allergy is clear. The results of the present study, in consideration of the relationship between tIgE values and number of sensitizations and the relationship between tIgE values and sIgE, showed weak correlations, respectively. Results from ROC analysis indicate that the capacity to distinguish between having/not having symptoms based on tIgE levels was low, and that this capacity to differentiate would be insufficient for diagnosis.

Our study had some limitations. We obtained data on pollen allergy symptoms solely from interviews and did not evaluate the relationship between the presence or absence of pollen allergy based on a doctor's diagnosis or the results of serum IgE testing. The patients had type I allergic disease, similar to allergic rhinitis, and there were no interview questions on whether or not they had asthma, atopic dermatitis, or other conditions with which allergen-sIgE

levels are related. CCDs were considered to have a false-positive effect on testing, but a limitation is the lack of consideration of whether or not they had entopy,[20] which has recently attracted attention as a cause of false negatives. Although useful data were obtained, there are no prior reports on the extent to which allergen-sIgE antibody measurement using serum is effective for pollen allergy screening, alongside interview questions regarding pollen allergy symptoms, in an analysis performed during medical examination of employees. Participants in the corporate medical examination were aged 19–68 years, with a mean age of 40.3 years, enabling analysis from adolescence to middle age, where allergic rhinitis is most common. None of the subjects were youths or older people aged 70 years or older. Though IgE levels reportedly decrease significantly at ages over 65 years for insect allergies, asthma, and allergic rhinitis, beyond atopic dermatitis,[21] there is no need to correct the data based on age. The blood samples were collected within a short period of 5 days, which was very suitable for data analysis for seasonal pollen allergy.

In conclusion, cedar pollen-sIgE values showed sufficient diagnostic performance for use in pollen allergy screening. Simultaneous measurement of tIgE levels had a low ability to differentiate between whether or not a pollen allergy was present, casting a doubt on whether it can be used to screen for pollen allergy. The study of CCDs suggested that subjects who tested positive for pollen often had false-positive results due to the impact of CCDs. Our study is the first to analyze the usefulness of these measurements, and we believe that these results can encourage the application of screening tests for pollen allergy in future health examinations.

ORCID iD

Hidenori Yokoi https://orcid.org/0000-0001-7058-3553

References

1. Bousquet J, Khaltaev N, Cruz AA, et al. Allergic rhinitis and its impact on asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy*. 2008;63(Suppl 86):8–160.
2. Lamb CE, Ratner PH, Johnson CE, et al. Economic impact of workplace productivity losses due to allergic rhinitis compared with select medical conditions in the United States from an employer perspective. *Curr Med Res Opin*. 2006;22:1203–1210.
3. Boulet LP, Turcotte H, Laprise C, et al. Comparative degree and type of sensitization to common indoor and outdoor allergens in subjects with allergic rhinitis and/or asthma. *Clin Exp Allergy*. 1997;27:52–59.
4. Yamada T, Saito H, Fujieda S. Present state of Japanese cedar pollinosis: the national affliction. *J Allergy Clin Immunol*. 2014;133:632–639. e5.
5. Bousquet J, Van Cauwenberge P, Bachert C, et al. Requirements for medications commonly used in the treatment of allergic rhinitis. European academy of allergy and clinical immunology (EAACI), allergic rhinitis and its impact on asthma (ARIA). *Allergy*. 2003;58:192–197.
6. Ikeda K, Yokoi H, Saito T, Kawano K, Yao T, Furukawa M. Effect of resection of the posterior nasal nerve on functional and morphological changes in the inferior turbinate mucosa. *Acta Otolaryngol*. 2008;128:1337–1341.
7. Berger WE, Nayak AS, Staudinger HW. Mometasone furoate improves congestion in patients with moderate-to-severe seasonal allergic rhinitis. *Ann Pharmacother*. 2005;39:1984–1989.
8. Krouse HJ. Environmental controls and avoidance measures. *Int Forum Allergy Rhinol*. 2014;4(Suppl 2):S32–S34.
9. Costongs GM, Bas BM. The first fully automated allergy analyser UniCAP: comparison with IMMULITE for allergy panel testing. *Eur J Clin Chem Clin Biochem*. 1997;35:885–888.
10. Rath N, Raje N, Rosenwasser L. Immunoglobulin E as a biomarker in asthma. *Immunol Allergy Clin North Am*. 2018;38:587–597.
11. Aalberse RC, Akkerdaas J, van Ree R. Cross-reactivity of IgE antibodies to allergens. *Allergy*. 2001;56:478–490.
12. Foetisch K, Westphal S, Lauer I, et al. Biological activity of IgE specific for cross-reactive carbohydrate determinants. *J Allergy Clin Immunol*. 2003;111:889–896.
13. Yokoi H, Yoshitake H, Matsumoto Y, et al. Involvement of cross-reactive carbohydrate determinants-specific IgE in pollen allergy testing. *Asia Pac Allergy*. 2017;7:29–36.
14. Okubo K, Kurono Y, Ichimura K, et al. Japanese guidelines for allergic rhinitis 2020. *Allergol Int*. 2020;69:331–345.

15. Bousquet J, Lockey R, Malling HJ. Allergen immunotherapy: therapeutic vaccines for allergic diseases. A WHO position paper. *J Allergy Clin Immunol*. 1998;102:558–562.

16. Yunginger JW, Ahlstedt S, Eggleston PA, et al. Quantitative IgE antibody assays in allergic diseases. *J Allergy Clin Immunol*. 2000;105:1077–1084.

17. Ollert M, Weissenbacher S, Rakoski J, Ring J. Allergen-specific IgE measured by a continuous random-access immunoanalyzer: interassay comparison and agreement with skin testing. *Clin Chem*. 2005;51:1241–1249.

18. Potaczek DP, Nasta Ek M, Wojas-Pelc A, Undas A. The relationship between total serum IgE levels and atopic sensitization in subjects with or without atopic dermatitis. *Allergol Int*. 2014;63:485–486.

19. Chung D, Park KT, Yarlagadda B, Davis EM, Platt M. The significance of serum total immunoglobulin E for in vitro diagnosis of allergic rhinitis. *Int Forum Allergy Rhinol*. 2014;4:56–60.

20. Powe DG, Bonnin AJ, Jones NS. 'Entopy': local allergy paradigm. *Clin Exp Allergy*. 2010;40:987–997.

21. Mediaty A, Neuber K. Total and specific serum IgE decreases with age in patients with allergic rhinitis, asthma and insect allergy but not in patients with atopic dermatitis. *Immun Ageing*. 2005;2:9.