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Relationship Between Airborne Pollen Counts and the Results Obtained Using 2 Diagnostic Methods: Allergen-Specific Immunoglobulin E Concentrations and Skin Prick Tests

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Abstract

Background: Patients with pollinosis show allergic symptoms related to airborne pollen levels, although this association is not always close. The use of new diagnostic techniques could improve our knowledge of this relationship.
Objective: To evaluate the relationship between pollen counts and the results obtained using 2 diagnostic techniques: the skin prick test and allergen-specific immunoglobulin E (sIgE) concentrations in serum.
Methods: Sixty-eight pollen-allergic patients were diagnosed using a combination of the high-capacity screening approach ADVIA Centaur® with a panel of 13 purified allergens and a skin prick test (SPT) with conventional extracts. Pollen levels were obtained by means of a volumetric sampler.
Results: The highest percentages of sensitization were detected for grass mixture allergens and major recombinant grass allergens (Phl p 1 and Phl p 5), followed by olive tree extracts and olive allergens (Ole e 1 and Ole e 9), in SPT and using recombinant allergens, respectively. The main pollen types registered in the atmosphere during 2006 and 2007 were *Quercus*, Poaceae, and Cupressaceae. A statistically significant correlation was observed between total pollen levels and median values of sIgE, especially in 2007.
Conclusion: A strong and significant positive correlation was found between pollen counts and sIgE levels. This correlation was weaker in the case of SPT and airborne pollen.

Key words: Airborne pollen. Specific IgE. SPT. Allergen.

Resumen

Antecedentes: Los pacientes con polinosis presentan síntomas alérgicos que no siempre se encuentran relacionados con los niveles de polen atmosférico. El uso de nuevos métodos para el diagnóstico de alergopatías puede contribuir a conocer mejor esta relación.
Objetivo: Evaluar la relación entre el contenido de polen atmosférico y dos métodos de diagnóstico, como son las pruebas intraepidérmicas y las concentraciones de IgE específica en el suero sanguíneo de los pacientes estudiados.
Métodos: El uso combinado del sistema de inmunoensayo ADVIA Centaur® con 13 alérgenos purificados y pruebas intraepidérmicas (PIE) con extractos de uso convencional en el diagnóstico de 68 pacientes que presentaron alergia a granos de polen, junto a los niveles de polen atmosférico obtenidos a través de un captador volumétrico.
Resultados: Los porcentajes de sensibilización más elevados que presentaron los pacientes fueron detectados para la mezcla de alérgenos de gramíneas y los mayores alérgenos recombinantes de gramíneas (Phl p 1 y Phl p 5), seguidos por extractos de alérgenos de olivo y alérgenos de olivo (Ole e 1 y Ole e 9), a través de pruebas intraepidérmicas y la utilización de alérgenos recombinantes, respectivamente. Los principales tipos de polen contabilizados en la atmósfera durante los años 2006 y 2007 fueron *Quercus*, Poaceae y Cupressaceae. Además, se encontró una correlación significativa entre los niveles de polen y la mediana de los valores de IgE específica, sobre todo en el año 2007.
Conclusión: Los niveles de polen en la atmósfera y de IgE específica en el suero sanguíneo presentaron una alta correlación significativa, menor en el caso de las PIE y los valores de polen atmosférico.

Palabras clave: Polen atmosférico. IgE específica. PIE. Alérgeno.

Introduction

The prevalence of pollen allergy is currently estimated to be 23% [1] and increasing [2]. Exposure to pollen allergens is a key factor among the environmental determinants of allergy symptoms, including air pollution [3]. Traditionally, methods for the measurement of allergen exposure have been based on counting pollen grains in air samples [4], reporting regional pollen loads with a major impact on the clinical presentation of seasonal respiratory allergy [5], and the results of skin prick tests (SPT) with batteries of pollen extracts. During the last few decades, the use of antibody-based allergen reaction systems [6] derived from enzyme-linked immunosorbent assays [7] has made it possible to evaluate allergen-specific immunoglobulin (Ig) E (sIgE) in serum and has provided critical information in patients with clinical allergy [8]. In these assays, the allergens are labeled with a ligand or immobilized to a solid phase. Solid phase technology has a series of inherent problems, with the result that new sIgE assays based on the presence of allergen extracts in a liquid phase have been developed, for example, the ADVIA Centaur immunoassay system (Bayer, Leverkusen, Germany) [9]. In addition, the advantages of this new assay include the use of a small quantity of serum (25 μL) in each test, no interference from IgG or high levels of nonspecific IgE, and high accuracy and specificity [10].

In recent years, studies on the possible relationship between pollen counts and symptoms [11,12] have revealed an increase in the presence of symptoms when pollen levels were higher. Some authors have examined the relationship between airborne pollen and the prevalence of positive SPT results [13] or sIgE levels in serum [14].

We analyzed the correlation between the SPT and allergen sIgE concentrations in serum. We based our analysis on the pollen counts of the main pollen types in order to evaluate sensitization to different pollen allergens in different samples using both methods and the possible correlation with pollen levels in 2 consecutive pollen seasons.

Material and Methods

Patients

Our study sample comprised patients from the Salamanca subsample of the EXPO2 study [18]. Briefly, 68 patients were consecutively selected if they fulfilled the following inclusion criteria: (i) a clinical history compatible with pollen allergy (rhinitis, rhinoconjunctivitis, or asthma) for at least the previous 2 years; (ii) no previous immunotherapy; and (iii) residence in Salamanca province (Spanish administrative unit) for at least the previous 5 years. Patients were evaluated out of the pollen season (September-December, 2006). The ethics committee approved the study.

Skin Prick Tests

SPTs were performed following established guidelines [15] using standard allergen extracts (*Artemisia vulgaris, Betula verrucosa, Cupressus arizonica, Olea europaea, Parietaria judaica, Plantago lanceolata, Platanus acerifolia,* polcalcin, profilin, *Salsola kali*, and a grass mixture with *Dactylis, Festuca, Lolium, Phleum*, and *Poa*), negative controls (saline), and positive controls (histamine) provided by ALK-Abelló S.A. (Madrid, Spain). The reactions were evaluated as positive when the diameter of the wheal was ≥3 mm after 20 minutes.

ADVIA Centaur Specific IgE Assay

The Advia Centaur specific IgE assay is a reverse sandwich immunoassay based on direct chemiluminescent technology [16]. The monoclonal mouse antihuman IgE antibody is covalently bound to paramagnetic particles (PMPs) in a solid phase. The anti-IgE coupled to the PMPs captures the sample IgE. Non-IgE is removed by washing. Biotinylated allergen is added in excess and binds to the allergen-specific IgE antibody captured on the solid phase; acridinium ester-labeled streptavidin in the LITE reagent binds to the biotin-labeled allergen. A direct relationship exists between the amount of allergen-specific IgE present in the sample and the amount of relative light units detected by the system. The allergens were a follows: *Artemisia vulgaris* (nArt v 1), *Betula verrucosa* (nBet v 1), *Cupressus sempervirens* (nCup s 1), grass (*Phleum pratense*, nPhl p 1, and nPhl p 5), *Olea europaea* (nOle e 1 and Ole e 9), *Parietaria judaica* (nPar j 1), *Plantago lanceolata* (nPla l 1), *Salsola kali* (nSal k 1), and panallergens (apple profilin rMal d 4, polcalcin from *Chenopodium album*, rChe a 3, and nonspecific lipid transfer protein (LTP) from peach [rPru p 3]). The detection limit of this system is 0.35 kUA/L, which is consistent with that of other in vitro methods to identify specific IgE [17]. Due to the broad range of specific IgE values, results were expressed as the median of specific IgE [18].

Pollen Sampling and Counts

Salamanca (40° 58′N, 5° 40′W) is situated in western Spain at 800 m above sea level. It has a Mediterranean continental climate [19] and a population of around 180 000 inhabitants. The city is surrounded by wide expanses of Mediterranean pasture to the south and arable farming land to the north. A 7-day Burkard volumetric recording trap was used in this study during the years 2006 and 2007. The trap was installed on the rooftop of a historical building in the city center, at 20 m above ground level. Sampling method, slide preparation, and data interpretation were performed according to the recommendations of the Spanish Aerobiology Network [20].

Statistical Analysis

Statistical analysis was performed with SPSS for Windows, version 12 (SPSS Inc., Chicago, Illinois, USA). The Spearman rank correlation and regression analysis were applied to evaluate the relationship between pollen counts and numbers of patients with a positive SPT result and ADVIA Centaur results. The correlation between pollen counts and sIgE values was assessed using total pollen counts and median sIgE for any of the studied allergens, except in the case of the panallergens Che a 3, Pru p 3, and Mal d 4, which were analyzed using the Spearman rank correlation and regression analysis. The degree of cross-reactivity between Ole e 1 and its homologous Fra e 1 [21] was the basis for evaluation of this allergen with the total

sum of *Olea* and *Fraxinus* pollen types in 2006 and 2007. As for SPT, in order to compare the prevalence of positive results and pollen counts, we used the percentage of all the pollen types studied over total pollen count.

Results

The 68 selected patients had a mean age of 30 years (Table 1); 81% were adults and almost 12% were aged 15-17 years. The clinical diagnoses were rhinitis and rhinoconjunctivitis (55.9%), followed by rhinitis and rhinoconjunctivitis plus asthma (42.6%), and asthma without nasal or ocular symptoms (1 patient). Only 1 patient presented serious and persistent asthma, whereas 31 showed the same severity with rhinitis. In addition, severity was mild or moderate and persistent in 63.3% of patients with asthma and in 38.8% of patients with rhinitis.

The most common positive SPT results for pollen allergen extracts were with grass mixture (at least 79.5%), followed by olive tree (42.6%), *Artemisia* (17.7%), *Salsola*, plane tree and Cupressaceae (16.2%), LTP (Pru p 3) of peach (11.8%), *Parietaria* (7.4%), polcalcin (5.9%), and birch (3%), although in this case the number of patients studied was 33.

The main sensitizations detected for recombinant or natural allergens (Table 2) were to Phl p 1, with almost 81%, and Phl p 5, with almost 46%. Ole e 1 and Ole e 9 were the allergens involved in 48.5% and 4.4% of sensitizations, respectively. Other important sensitizations were to Cup s 1 (19.1%), Pla l 1 (14.7%), Art v 1 (13.3%), Sal k 1 (8.8%), and Par j 1 and Bet v 1 (4.4%). In the case of panallergens, these percentages were 13.2% to LTP (Pru p 3), almost 12% to profilin (Mal d 4), and 4.4% to polcalcin (Che a 3).

The sIgE values obtained with ADVIA Centaur, expressed as the median, varied widely depending on the allergen studied (Table 2). The grass allergens Phl p 5 and Phl p 1, with 14.5 and 10.8 IU/mL, respectively, showed the highest sIgE values, followed by the panallergen polcalcin (Che a 3) with 8.1 IU/mL and the olive tree allergens Ole e 1 and Ole e 9, with 6.1 and 1.3 IU/mL, respectively. The other allergens, organized according to their sIgE values, were Pla l 1 (4.2 IU/mL), Cup s 1 and Sal k 1 (almost 3 IU/mL), Pru p 3 (2.5 IU/mL), Bet v 1 and Art v 1 (almost 2 IU/mL), and profilin Mal d 4 (1.3 IU/mL) and Par j 1 (1 IU/mL).

A total of 25 744 and 31 103 pollen grains were registered in the atmosphere of Salamanca during the years 2006 and 2007. The most important types of pollen detected in both years were *Quercus* (23.1% and 30.1% of the annual total, respectively), Poaceae (20.2% and 25.2%), and Cupressaceae (9.5% and 11.7%), followed by *Olea* (7.6%), *Platanus* (5.9%), and Urticaceae (4.6%) in 2006, and *Plantago* (5.5%), *Populus* (3.5%), and *Platanus* (3.3%) in 2007 (Table 3).

Table 1. Demographic and Clinical Results of the Study Patients

Demographic Results					
		Mean (SD)		Minimum	Maximum
Age,		29.5	(10.7)	11	63
Evolution, y		7	(5.9)	1	25
		No.	(%)		
Age, y	≤4	5	(7.3)		
	15-17	8	(11.8)		
	≥18	55	(80.9)		
Sex	Women	38	(55.9)		
	Men	30	(44.1)		
Clinical Results					
			No.	(%)	
Diagnosis	Asthma		1	(1.5)	
	Rhinitis/ Rhinoconjunctivitis		38	(55.9)	
	Rhinitis/ Rhinoconjunctivitis + Asthma		29	(42.6)	
Severity of asthma	Intermittent		10	(33.3)	
	Mild persistent		10	(33.3)	
	Moderate persistent		9	(30)	
	Severe persistent		1	(3.3)	
Severity of rhinitis	Intermittent		10	(14.9)	
	Mild persistent		19	(28.4)	
	Moderate intermittent		7	(10.4)	
	Severe persistent		31	(46.3)	

Table 2. Specific Immunoglobulin E Values and Number of Positive Results and Prevalence Obtained With the ADVIA Centaur Assay and Skin Prick Test[a]

Specific Inmunoglobulin E				Skin Prick Test		
Allergen	Median	Positive	Prevalence, %	Allergen	Positive	Prevalence, %
Art v 1	1.7	9	13.2	*Artemisia*	12	17.7
Bet v 1	1.8	3	4.4	*Betula*[b]	1	3
Cup s 1	2.8	13	19.1	*Cupressus*	11	16.2
Ole e 9	1.3	3	4.4	Grasses	54	79.4
Ole e 1	6.1	33	48.5	*Olea*	29	42.6
Par j1	1	3	4.4	Peach	8	11.8
Phl p 1	10.8	55	80.9	*Parietaria*	5	7.4
Phl p 5	14.5	31	45.6	*Platanus*	11	16.2
Pla l 1	4.2	10	14.7	*Plantago*	24	35.3
Che a 3	8.1	3	4.4	Polcalcin	4	5.9
Mal d 4	1.3	8	11.8	Profilin	11	16.2
Pru p 3	2.5	9	13.2	*Salsola*	11	16.2
Sal k 1	2.6	6	8.8			

[a]N=68, values expressed as median IU/mL
[b]In this case, n=33

Table 3. Main Pollen Types Related to Pollen Allergy and Their Annual Total in the Years 2006 and 2007 With Percentages Over Total Airborne Pollen Counted in Each Year

	2006		2007	
Pollen Type	%	Annual Pollen, Grains	%	Anual Pollen, Grains
Artemisia	0.3	76	0.2	67
Betula	0.5	137	0.5	150
Chenopodiaceae	1.8	474	0.7	228
Cupressaceae	9.5	2438	11.7	3635
Olea	7.6	1954	2.2	678
Plantago	3.4	868	5.5	1706
Platanus	5.9	1530	3.3	1017
Poaceae	20.2	5210	25.2	7832
Urticaceae	4.6	1197	3.2	1002

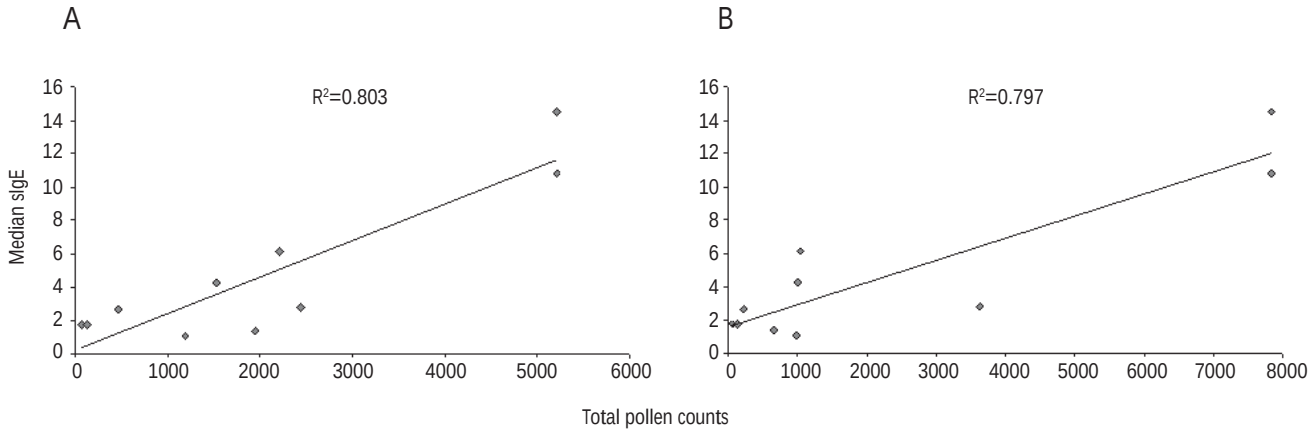

Figure 1. Relationship between median serum specific immunoglobulin E values (in IU/mL) and total annual pollen counts of related pollen types during the years 2006 (A) and 2007 (B).

Table 4. Statistical Analysis by Means of Regression Analysis and Spearman Rank Correlation of Pollen Counts and the 2 Diagnostic Methods Used

	Regression Analysis [y=b1x+c]							Nonparametric Analysis
	Year	R^2	F	df	Sig	Const	Coef. b1	Spearman corr. coef.
Median sIgE – total pollen	2006	0.80	32.614	9	.001	0.2069	0.0022	0.717[a]
	2007	0.80	31.409	9	.001	1.5675	0.0013	0.766[b]
% SPT – % pollen type	2006	0.76	22.177	8	.002	0.0242	0.2286	0.542
	2007	0.58	9.659	8	.0017	-0.9	0.2609	0.339

Abbreviations: Coef. b1, coefficient b1; Const., constant; df, degrees of freedom; F, mean squares statistic; R2, determination index; sIgE, specific immunglobulin E; Sig., significance; Spearman corr. coef.: Spearman correlation coefficient.
[a]Significance set at 95%.
[b]Significance set at 99%.

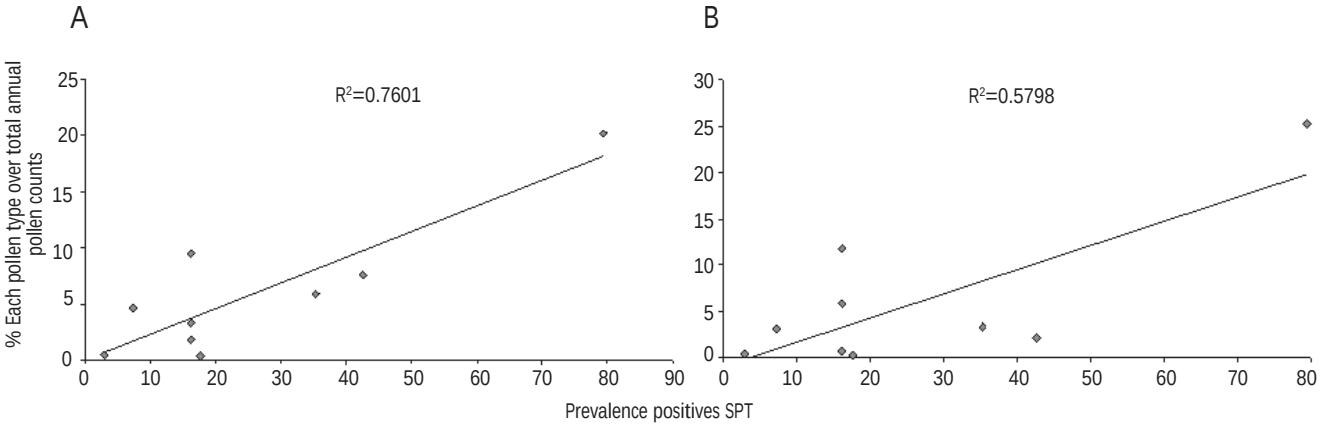

Figure 2. Relationship between the prevalence of positive results with skin prick tests and the percentage of each related pollen type over total airborne pollen registered during the years 2006 (A) and 2007 (B).

The correlation coefficients of the Spearman rank correlation test for 2006 and 2007 (Figure 1, Table 4) showed a positive and significant correlation between total pollen counts and median sIgE values during both years, a relationship supported by regression analysis and its determination index. A lower positive correlation between percentage of total pollen counts and prevalence of SPT was registered by means of the 2 statistical methods used (Figure 2, Table 4).

Discussion

Pollen-allergic patients in the city of Salamanca were predominantly sensitized to grass pollen allergens and to olive pollen allergens, as described in other studies carried out in towns in the center of the Iberian Peninsula [22,23]. In general terms, the prevalence of positive SPT results with different pollen (grass mixture, *S kali,* and *P judaica*) in 12 Spanish cities [24] was similar in towns located in the middle and in the west of Spain. This prevalence varies with the geographic area, eg, *O europaea* is much more prevalent in the south and south-west [25,26], and could be due to annual differences or even the presence of false positive or negatives resulting from cross-reactivity among allergens [27].

The relationship between sIgE levels for any allergen, excluding panallergens, and the annual total of each related pollen type, including total sums of *Fraxinus* and *Olea* for Ole e 1, were higher than the relationship registered between prevalence of positive SPT and percentages of each type of pollen over annual pollen counts, especially in 2007. Other authors reported similar results with no correlation between pollen levels and positive SPT results, as occurred in a study in Ankara (Turkey) [28] in which the authors detected many positive results for grass pollen allergens in SPT and low levels of airborne grass pollen counts, or in Ohio [29], where no significant correlation between airborne pollen from trees and prevalence in SPT was observed.

Few studies report the relationship between pollen counts and pollen allergen levels or even between airborne pollen and sIgE levels in serum. A study in Cartagena (southeast Spain) [30] revealed a statistically significant correlation between the levels of pollen allergens (Ole e 1, Par j 1, and Par j 2) in the atmosphere and pollen counts, whereas in a study performed in Gdansk (Poland) [31], no correlation was observed between

grass pollen allergen and pollen counts (high levels of sIgE for grasses in serum and low airborne grass pollen counts). This lack of correlation could be due to the use of a gravimetric sampler, because the results obtained with this sampler differ from those obtained with a volumetric spore trap [32,33].

One of the limitations of our study was that it was performed over only 1 year. In addition, we did not take into account the presence of aeroallergens outside the pollen season [34] or the influence of other meteorological phenomena, such as storms, and their known effect on the increase in allergen counts [35].

The relationship between allergen sensitization and allergen load is well documented [36]. In our study, the correlation between sIgE levels in serum and pollen counts could indicate that sIgE levels reflect higher antigenic load exposure, as occurred in other works displaying high counts of airborne latex particles and high levels of latex sIgE [37,38].

In conclusion, these results showed that specific IgE levels to single allergen components had a better correlation with pollen loads of related pollen types than the correlation between SPT results and airborne pollen counts.

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