

Predictive Capabilities of Serum Eosinophil Cationic Protein, Percentage of Eosinophils and Total Immunoglobulin E in Allergic Rhinitis without Bronchial Asthma

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This study identified cut-off values for allergy markers for use in the diagnosis of allergic rhinitis in the absence of other allergic diseases. Total immunoglobulin E (IgE), eosinophil cationic protein (ECP) and the numbers of eosinophils were measured in serum samples from 442 patients with typical symptoms of allergic rhinitis. A definite diagnosis was made on the basis of the presence of specific IgE levels. Cut-off values with a maximal discrimination to diagnose allergic rhinitis were found to be 98.7 IU/ml, 24.7 µg/ml and 4.0% for total IgE, ECP and eosinophils, respectively.

Sensitivity, specificity and odds ratio for these values were 75.2%, 69.7% and 6.93, respectively, for total IgE, 55.7%, 74.4% and 3.70 for ECP, and 57.5%, 72.0% and 3.47 for eosinophils. A composite score representing positive results for all three markers had a positive predictive value of 85.3%, with an odds ratio of 8.55. It was concluded that total serum IgE, ECP and eosinophil percentage are strong predictors of allergic rhinitis and the determination of cut-off values for these markers can aid in the diagnosis of allergic rhinitis in the clinical setting.

KEY WORDS: ALLERGIC RHINITIS; BIOLOGICAL MARKER; EOSINOPHIL CATIONIC PROTEIN; EOSINOPHIL; IMMUNOGLOBULIN E

Introduction

Allergic rhinitis is a common condition: its current prevalence is between 17.0% and 28.5% and is increasing.^{1,2} The association between allergic rhinitis and allergic asthma increases the importance of diagnosing allergic rhinitis accurately. Diagnosis of allergic rhinitis is made on the basis of a history of typical allergic symptoms (sneezing, itching and watery rhinorrhoea)

and the detection of specific immunoglobulin E (IgE) using *in vivo* tests (such as skin prick or intradermal tests), or *in vitro* tests (such as the radioallergosorbent test, multiple radioallergosorbent test or a fluorescence enzyme immunoassay).

Allergic rhinitis occurs primarily as a result of an IgE-mediated inflammatory reaction and eosinophil infiltration following exposure of the nasal mucosa to an allergen.

Since eosinophilic airway inflammation is a prominent feature of allergic rhinitis, the percentage of eosinophils in the serum, the total serum IgE and the serum levels of granular proteins derived from activated eosinophils such as eosinophil cationic protein (ECP) are considered to be important markers of this condition.^{3,4} Although the diagnosis of allergic rhinitis is made on a characteristic history and the detection of specific IgE, many studies have investigated the diagnostic value of allergy markers released during an allergic reaction.⁵⁻¹¹

Total IgE, percentage of eosinophils and ECP are well-known allergy markers that can easily be measured using simple serological tests. They are often measured in atopic diseases, particularly asthma. A number of studies have investigated the application of allergy marker cut-off values and concluded that they are useful in the diagnosis of allergic asthma.^{8,10} Little has been reported on the value of these markers in the diagnosis of allergic rhinitis. Some studies have reported that these allergy markers are associated with symptom severity in allergic rhinitis,^{4,6} though not all studies agree.^{12,13} The allergy marker cut-off values used in these studies are not clear and, as the sample sizes in most reports are small, the results are difficult to apply to the clinical setting. Some studies have suggested a reference value for allergy markers for use in the diagnosis of asthma^{6,10} and statistical parameters (such as sensitivity, specificity, and positive and negative predictive values) have been reported. In comparison with the lower respiratory tract, the nose is a relatively small organ. Thus, the cut-off values for allergy markers in allergic rhinitis may be different to those in asthma. To date, no suggested reference values for total IgE, percentage of eosinophils and ECP markers in serum for predicting allergic rhinitis have

been published.

The present study aimed to identify the cut-off values for total IgE, ECP and the percentage of eosinophils in serum samples that give appropriate sensitivity and specificity for use in the diagnosis of allergic rhinitis, in the absence of other allergic diseases such as asthma. The predictive capabilities of each allergy marker were compared, and the correlations between levels of these allergy markers and symptoms of allergic rhinitis were investigated.

Patients and methods

STUDY POPULATION

Patients from the Department of Otorhinolaryngology – Head and Neck Surgery, Samsung Medical Centre, Seoul, Republic of Korea, with a possible diagnosis of allergic rhinitis, were enrolled sequentially in this prospective study between May 2007 and July 2008. All patients had a history of two or more of the three major symptoms of allergic rhinitis (itching, sneezing and watery rhinorrhoea) for > 3 months. Patients with chronic paranasal sinusitis without nasal polyps or nasal septal deviation were included in the study, but those diagnosed with asthma, allergic dermatitis, a history of wheezing, sinusitis with nasal polyps or parasitic infections were excluded.

Verbal informed consent was obtained from all patients and the study protocol was approved by the Institutional Ethics Committee of Samsung Medical Centre.

CLINICAL AND LABORATORY EVALUATIONS

A score was obtained for each patient for each allergic rhinitis symptom (watery rhinorrhoea, itching and sneezing) using a scale of 1 (no discomfort) to 7 (maximal discomfort). These values were added together to give an overall symptom score. A skin prick

test or fluorescence enzyme immunoassay was performed to determine the presence of specific IgE, confirming the diagnosis of allergic rhinitis. Blood samples were taken at enrolment for the measurement of eosinophils, ECP and total IgE in serum. The percentage of eosinophils was measured through a differential peripheral blood count.

Skin prick tests

Skin prick tests were performed for 53 inhalant allergens (including *Dermatophagoides pteronyssinus*, *D. farinae*, common tree pollens, grass pollen, weed pollen, cat, dog and cockroach) following the manufacturer's guidelines (Allergy Therapeutics plc, Worthing, UK). No patient had taken antihistamines of any type, or any other medication that could influence the results, for 7 days prior to the test. Allergen extracts along with the appropriate negative (normal saline) and positive (1 mg/ml histamine) controls were applied on the upper back using the skin prick technique. A wheal 3 mm larger than that of the negative control, 15 min after pricking, was considered to be a positive allergic response. A wheal in response to any one of the allergens tested was considered to be a positive response.

Fluorescence enzyme immunoassay

Blood samples (3 ml) were allowed to clot and were then centrifuged at 2500 rpm for 5 min (Kubota 4000; Kubota Manufacturing, Fujioka, Japan). Serum samples were stored immediately at -20°C until testing. Specific IgE was measured in serum for six major inhaled allergens (*D. pteronyssinus*, *D. farinae*, cat, dog, tree mixture and weed mixture) using the ImmunoCAP® Allergy and Asthma Assay (Phadia [Thermo Fisher Scientific], Uppsala, Sweden). Assays were performed simultaneously according to the manufacturer's standard protocol. Results

were scored from 0 to 6 as follows: 0, ≤ 0.35 IU/ml; 1, 0.36 – 0.69 IU/ml; 2, 0.70 – 3.49 IU/ml; 3, 3.50 – 17.49 IU/ml; 4, 17.50 – 49.99 IU/ml; 5, 50.0 – 99.9 IU/ml; and 6, 100 IU/ml. A score ≥ 2 was considered positive. To reduce any day-to-day bias, assays were calibrated daily using a standard ImmunoCAP®-specific IgE control, according to the manufacturer's instructions. The ImmunoCAP® assay was also used to measure total IgE and ECP levels in serum.

STATISTICAL ANALYSES

Statistical analyses were performed using SPSS® statistical software, version 12 (SPSS Inc., Chicago, IL, USA) for Windows®. Data are reported as mean $\pm$ SD values. The least-significant *P*-value method was employed to calculate the cut-off values with the maximal discrimination between allergic and non-allergic patients for total IgE, ECP and percentage of eosinophils in serum. Sensitivity, specificity, odds ratios, and positive and negative predictive values were calculated using these cut-off values.

Correlations between the sum of the three allergy symptom scores and each allergy marker were calculated using Spearman's rank correlation coefficient. An independent *t*-test was used to compare age and allergy marker levels in the allergic and non-allergic groups. Gender distribution was compared using the χ^2 -test.

Cross-table analysis was used to compare sensitivities, specificities and odds ratios among the allergy markers. The area under the curve (AUC) for each parameter was calculated using receiver operating characteristic curve analysis to determine their predictive capabilities. Logistic regression with multivariate analysis was used for verification of the cut-off values and odds ratios. A value of $P < 0.05$ was considered to be statistically significant.

Results

In total, 442 patients (289 males, 153 females) with a median age of 33 years (range 8 – 76 years) were enrolled in the study. Both the skin prick test and the fluorescence enzyme immunoassay were carried out in 393 patients. In 23 patients, only the skin prick test was carried out and in 26 patients only immunoassay was used. Patient characteristics and allergy marker levels in serum samples from the allergic and non-allergic groups are given in Table 1. In total, 221 (50%) patients were positive for specific IgE on skin prick tests or fluorescence enzyme immunoassays, and were considered to have allergic rhinitis. There were no significant differences in age or gender distribution between the two groups. Total IgE ($P < 0.001$), serum ECP ($P < 0.001$) and percentage of eosinophils ($P = 0.015$) were all significantly increased in the allergic group compared with the non-allergic group. The AUC for total IgE was higher than the AUC for the other two parameters (Fig. 1, Table 2).

The cut-off values showing maximal discrimination were calculated to be 98.7 IU/ml for total IgE, 24.7 µg/ml for serum ECP and 4.0% for eosinophils. Patient distribution

according to these cut-off values is given in Table 3. The diagnostic power of each allergy marker using these cut-off values is shown in Table 4. The sensitivity of total IgE was significantly greater than that for serum ECP or percentage of eosinophils ($P < 0.001$), but there were no significant differences in specificity among the allergy markers (Table 4). The odds ratio calculated using the cut-off values was highest for total IgE (6.93, 95% confidence intervals [CI] 4.19, 9.62; $P < 0.001$); the odds ratio for serum ECP was 3.70 (95% CI 1.81, 6.32; $P = 0.001$), and for eosinophils was 3.47 (95% CI 1.26, 7.93; $P = 0.043$).

Each allergy marker showed a statistically significant correlation with the symptom score. Correlation coefficients were 0.167, 0.110 and 0.209 for total IgE, ECP and percentage of eosinophils, respectively, in serum.

In 95 patients (21.5%), levels of all three markers were higher than the respective cut-off values. Among this group, 81 patients were diagnosed with allergic rhinitis. The positive predictive value of the composite score when all three allergy markers were positive was 85.3% with an odds ratio of 8.55. In contrast, 118 patients (26.7%) were

TABLE 1:
Demographic data and allergy marker levels in serum samples from patients with symptoms of allergic rhinitis, divided according to the presence (allergic group) or absence (non-allergic group) of specific immunoglobulin E (IgE)

Parameter	Allergic group (<i>n</i> = 221)	Non-allergic group (<i>n</i> = 221)	Statistical significance
Age, years	27.9 ± 13.7	33.9 ± 16.3	NS ^a
Sex			NS ^b
Male	148	141	
Female	73	80	
Total IgE, IU/ml	468.6 ± 733.4	118.4 ± 180.8	$P < 0.001^a$
ECP, µg/ml	35.4 ± 40.2	21.9 ± 19.6	$P < 0.001^a$
Eosinophils, %	4.8 ± 3.0	3.2 ± 2.6	$P = 0.015^a$

Data presented as mean ± SD or number (*n*) of patients.

ECP, eosinophil cationic protein; NS, no statistically significant differences ($P > 0.05$).

^aCompared using an independent *t*-test; ^bcompared using the χ^2 -test.

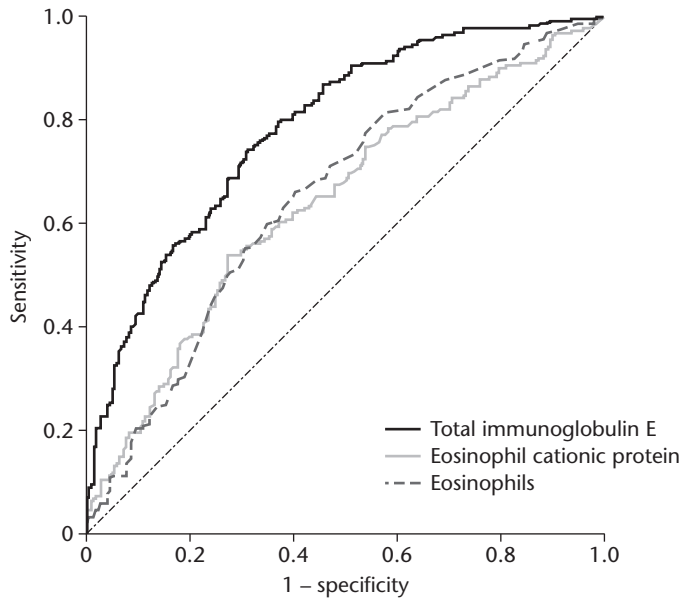


FIGURE 1: Receiver operating characteristic curves for serological allergy marker cut-off values in patients with symptoms of allergic rhinitis. The dotted and dashed line (area under the curve = 0.5) represents the chance predictor

negative for all three markers; among this group, 15 patients were diagnosed with allergic rhinitis. The negative predictive value of the composite score when all three markers were negative was 87.9% with an odds ratio of 12.0.

Discussion

The three popular allergy markers – total IgE, ECP and percentage of eosinophils in

serum – analysed in the present study, showed good predictive value for allergic rhinitis. The AUC for total IgE, along with the positive and negative predictive values, provided strong evidence of the value of total IgE in the diagnosis of this condition. Although the AUC values for serum ECP and percentage of eosinophils were lower than that for total IgE, they were also sufficient to be useful clinical tools. These results are

TABLE 2:
Area under the curve (AUC) for allergy markers in serum samples from patients with symptoms of allergic rhinitis

Allergy marker	AUC	SE	Statistical significance ^a	95% confidence intervals	
				Minimum	Maximum
Total IgE	0.787	0.021	$P < 0.001$	0.743	0.827
ECP	0.640	0.026	$P < 0.001$	0.588	0.691
Eosinophils	0.653	0.026	$P < 0.001$	0.602	0.704

^aArea under the receiver operating characteristic curve analysis.
IgE, immunoglobulin E; ECP, eosinophil cationic protein; SE, standard error.

TABLE 3:
Distribution of patients with (allergic group) or without (non-allergic group) specific immunoglobulin E according to serological allergy marker cut-off values

Allergy marker cut-off	Allergic group (n = 221)	Non-allergic group (n = 221)	Total
Total IgE	166	67	233
≥ 98.7 IU/ml			
< 98.7 IU/ml	55	154	209
ECP	123	56	179
≥ 24.7 µg/ml			
< 24.7 µg/ml	98	165	263
Eosinophils	127	62	189
≥ 4.0%			
< 4.0%	94	159	253

Data presented as number (n) of patients.

IgE, immunoglobulin E; ECP, eosinophil cationic protein.

TABLE 4:
Diagnostic power of the allergy markers at cut-off values in serum samples of 98.7 IU/ml for immunoglobulin E (IgE), 24.7 µg/ml for eosinophil cationic protein (ECP) and 4.0% for eosinophils from patients with symptoms of allergic rhinitis

Parameter	Total IgE	ECP	Eosinophils
Sensitivity, %	75.2 ^a	55.7	57.5
Specificity, %	69.7	74.4	72.0
Odds ratio	6.93	3.70	3.47
Positive predictive value, %	71.3	68.8	67.2
Negative predictive value, %	73.7	62.8	62.9

^aP < 0.001 versus ECP and eosinophils.

especially valuable due to the exclusion of patients with asthma, atopic dermatitis and other diseases related to an elevated IgE level in this study. Associations between the allergy markers and symptom severity were, however, relatively weak.

Other studies have found correlations between allergy markers and allergy status or symptom scores. Sin *et al.*¹⁴ reported that serum ECP and total IgE were higher in allergic patients than in non-allergic patients. In a study by Winther *et al.*,⁴ total IgE, ECP and eosinophil counts in serum were higher in patients diagnosed with allergic rhinitis compared with non-allergic patients. These studies showed a positive correlation

between allergy markers and allergic rhinitis, but they did not disclose the predictive value of the elevated allergy markers.^{4,14} The present study is the first to report the exact predictive powers of allergy markers in diagnosing allergic rhinitis. The sensitivity and specificity for total IgE were 75.2% and 69.7%, respectively; for serum ECP and percentage of eosinophils, the sensitivities were lower though comparable with each other (55.7% and 57.5%, respectively) and the specificities were only very slightly higher than for total IgE. Although the diagnosis of allergic rhinitis cannot be definitively made on the basis of allergy marker levels following serological analyses, these markers can

provide objective support.

The sensitivities and specificities of the allergy markers reported in the present study were relatively low, with sensitivities ranging from 55.7% to 75.2% and specificities ranging from 69.7% to 74.4%. A study in patients with asthma reported sensitivities of only 51% and 62% for serum ECP and eosinophils, respectively.¹⁰

The mean value for total IgE in the non-allergic group was 118 IU/ml, which is greater than the cut-off value. This group included a number of patients with high total IgE levels in the absence of evidence of allergic disease or other diseases associated with elevated IgE, resulting in a large standard deviation for the mean total IgE value. These patients were not omitted from the analysis, in order to reflect the clinical setting adequately.

The present study demonstrated a significant correlation between each allergy marker and the symptom score, but the correlation coefficients were relatively low, so preventing the conclusion that a strong clinical correlation between allergy markers and symptom scores could be found. The relationship between symptom severity and allergy markers remains controversial. Some research has reported a positive correlation between serum ECP levels and symptom scores;¹⁵ others have reported no such correlation, however.¹⁶ The clinical setting for the present study was a tertiary care hospital, so complaints about allergy symptoms may have been higher in the patient pool, irrespective of allergy status. If the same study were to be performed at a primary care clinic, a stronger correlation between allergy markers and symptom scores might be identified. In addition, the present study was conducted over a 14-month period and so may be affected by seasonal variations in allergy markers,

although such changes have been reported to be negligible.⁴

A number of studies have described the clinical value of allergy markers in bronchial asthma. Niimi *et al.*¹⁷ found that the serum ECP level reflected eosinophilic airway inflammation and asthma activity. Shields *et al.*¹⁰ reported that serum ECP levels and percentages of blood eosinophils might be useful markers for predicting eosinophilic airway inflammation in wheezing children, with suggested cut-off values. To date, however, no studies have determined the cut-off values for allergy markers in the diagnosis of allergic rhinitis without asthma.

Allergic rhinitis is sometimes linked with allergic asthma, and allergy markers have also been measured in asthmatic patients.¹⁸ Based on this, the importance of these markers cannot be underestimated.

In conclusion, the present study is the first to determine cut-off values for serum allergy markers for the diagnosis of allergic rhinitis. Total serum IgE and ECP levels, and eosinophil percentages, were shown to be strong predictors of allergic rhinitis in the clinical setting. The sensitivity of total IgE was higher than that of the other two allergy markers, although there was little difference in specificity. A composite score, including all three markers, had strong positive and negative predictive values. Further studies examining the levels of allergy markers in nasal secretions would be of value.

Acknowledgement

This study was sponsored by the Hyoseok Research Fund, Samsung Changwon Hospital, Changwon, Republic of Korea.

Conflicts of interest

The authors had no conflicts of interest to declare in relation to this article.

• Received for publication 30 March 2011 • Accepted subject to revision 9 August 2011

• Revised accepted 5 October 2011

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References

- 1 Bauchau V, Durham SR: Prevalence and rate of diagnosis of allergic rhinitis in Europe. *Eur Respir J* 2004; **24**: 758 – 764.
- 2 Bauchau V, Durham SR: Epidemiological characterization of the intermittent and persistent types of allergic rhinitis. *Allergy* 2005; **60**: 350 – 353.
- 3 Droste JH, Kerhof M, de Monchy JG, *et al*: Association of skin test reactivity, specific IgE, total IgE, and eosinophils with nasal symptoms in a community-based population study. The Dutch ECRHS Group. *J Allergy Clin Immunol* 1996; **97**: 922 – 932.
- 4 Winther L, Moseholm L, Reimert CM, *et al*: Basophil histamine release, IgE, eosinophil counts, ECP, and EPX are related to the severity of symptoms in seasonal allergic rhinitis. *Allergy* 1999; **54**: 436 – 445.
- 5 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, GA²LEN and AllerGen). *Allergy* 2008; **63**(suppl 86): 8 – 160.
- 6 Chen ST, Sun HL, Lu KH, *et al*: Correlation of immunoglobulin E, eosinophil cationic protein, and eosinophil count with the severity of childhood perennial allergic rhinitis. *J Microbiol Immunol Infect* 2006; **39**: 212 – 218.
- 7 Cheng KJ, Wang SQ, Xu YY, *et al*: Serum ECP levels in patients with allergic rhinitis and chronic rhinosinusitis. *Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi* 2009; **44**: 1001 – 1005 [in Chinese, English abstract].
- 8 Remes S, Korppi M, Remes K, *et al*: Serum eosinophil cationic protein (ECP) and eosinophil protein X (EPX) in childhood asthma: the influence of atopy. *Pediatr Pulmonol* 1998; **25**: 167 – 174.
- 9 Selnes A, Dotterud LK: No association between serum eosinophil cationic protein and atopic dermatitis or allergic rhinitis in an unselected population of children. *J Eur Acad Dermatol Venereol* 2005; **19**: 61 – 65.
- 10 Shields MD, Brown V, Stevenson EC, *et al*: Serum eosinophilic cationic protein and blood eosinophil counts for the prediction of the presence of airways inflammation in children with wheezing. *Clin Exp Allergy* 1999; **29**: 1382 – 1389.
- 11 Yüksel B, Aydemir C, Ustündag G, *et al*: The effect of treatment with montelukast on levels of serum interleukin-10, eosinophil cationic protein, blood eosinophil counts, and clinical parameters in children with asthma. *Turk J Pediatr* 2009; **51**: 460 – 465.
- 12 Dykewicz MS, Fineman S: Executive Summary of Joint Task Force Practice Parameters on Diagnosis and Management of Rhinitis. *Ann Allergy Asthma Immunol* 1998; **81**: 463 – 468.
- 13 Nickelsen JA, Georgitis JW, Reisman RE: Lack of correlation between titers of serum allergen-specific IgE and symptoms in untreated patients with seasonal allergic rhinitis. *J Allergy Clin Immunol* 1986; **77**: 43 – 48.
- 14 Sin A, Terzioğlu E, Kokuludağ A, *et al*: Serum eosinophil cationic protein (ECP) levels in patients with seasonal allergic rhinitis and allergic asthma. *Allergy Asthma Proc* 1998; **19**: 69 – 73.
- 15 Hsu PY, Yang YH, Lin YT, *et al*: Serum eosinophil cationic protein level and disease activity in childhood rhinitis. *Asian Pac J Allergy Immunol* 2004; **22**: 19 – 24.
- 16 Yüksel H, Yılmaz O, Sogut A, *et al*: Correlation of quality of life with clinical parameters and eosinophilic cation protein levels in children with allergic rhinoconjunctivitis. *Int Arch Allergy Immunol* 2009; **148**: 18 – 22.
- 17 Niimi A, Amitani R, Suzuki K, *et al*: Serum eosinophil cationic protein as a marker of eosinophilic inflammation in asthma. *Clin Exp Allergy* 1998; **28**: 233 – 240.
- 18 King MJ, Bukantz SC, Phillips S, *et al*: Serum total IgE and specific IgE to *Dermatophagoides pteronyssinus*, but not eosinophil cationic protein, are more likely to be elevated in elderly asthmatic patients. *Allergy Asthma Proc* 2004; **25**: 321 – 325.

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