



Association of serum eosinophilia and total immunoglobulin E concentration with the risk of allergic symptoms and allergic sensitization, respectively: A 2-year follow-up study

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ABSTRACT

Objectives: Serum eosinophil percentage (SEP), eosinophil cationic protein (ECP) concentration, and total IgE (TlgE) concentration are known to increase in patients with allergic disease; however, the relevance of these elevated markers in children with negative allergic test results remains unclear. Therefore, this 2-year follow-up study aimed to investigate whether SEP, ECP concentration, and TlgE concentration are associated with an increased risk for allergic sensitization and allergic symptoms.

Methods: In 2012, SEP, ECP concentration, and TlgE concentration of third- and fourth-grade students from 6 elementary schools were measured. Skin prick test was performed, and the presence of allergic nasal symptoms was investigated using a questionnaire. In 2014, the same examinations were performed in the same set of students, who were then in the fifth and sixth grades, respectively, of the same schools. The cut-off value and usefulness of serologic markers (eosinophil count, ECP concentration, and TlgE concentration) were calculated using the receiver operating characteristic curve.

Results: Serum eosinophil percentage (cut-off value, 3.8%) was associated with the newly developed allergic nasal symptoms (sensitivity, 77.9; specificity, 41.8). A high serum TlgE concentration (cut-off value, 17.7 IU/mL) was also associated with the risk for allergic sensitization (sensitivity, 46.3; specificity, 85.3).

Conclusion: SEP and TlgE concentration were associated with the development of allergic symptoms and allergic sensitization, respectively, 2 years after the first examination.

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1. Introduction

Recently, the prevalence of allergic rhinitis (AR) has been steadily rising [1]. Various in vitro and in vivo tests have been used for AR diagnosis. Among the in vivo tests, skin prick tests (SPTs) and intradermal tests have been widely used. In vitro tests to measure specific IgE concentrations, such as multiple allergen simultaneous test, radioallergosorbent test, and ImmunoCAP (Phadia, Uppsala, Sweden), have been used to diagnose allergic diseases. Previous

studies showed that total immunoglobulin E (TlgE) concentration, serum eosinophil count, and serum eosinophil cationic protein (ECP) concentration increased in patients with AR [2,3]. However, the diagnostic value of these markers for allergic sensitization is unknown.

In practice, we often encounter cases of patients with negative results of allergic tests, such as SPTs and in vitro tests, but with elevated TlgE concentration. Moreover, serum eosinophil count and ECP concentration could be elevated in persons with negative allergic test results [4,5]. Thus, the clinical significance of these serologic test results remains unclear. Especially, previous studies showed that the results of allergic tests could alter with time [6,7]. However, specific tests or markers that can individually predict the risk for allergic sensitization or symptoms are lacking.

Therefore, this 2-year follow-up study aimed to investigate whether eosinophil percentage, ECP concentration, and TlgE concentration were associated with the risk for allergic sensitization and allergic symptoms.

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2. Materials and methods

2.1. Study population

The initial study population in October 2012 consisted of third- and fourth-grade children from 6 elementary schools in Jeju Island, Korea. The details of the study were explained to the students and their parents, and written informed consent was obtained from their legal guardians. A questionnaire was distributed, filled out by the students and parents, and collected. SPTs and serologic tests (serum eosinophil percentage [SEP], eosinophil cationic protein concentration [ECP], and TlgE concentration) were performed on direct visit to each school. Two years later, the same investigations were conducted in October 2014 in the same set of children who were then in the fifth and sixth grades, respectively, of the same 6 elementary schools. In total, 720 children participated in the study in 2012, and 691 children, in 2014. Also, we investigated the medication history during 7 days before skin prick test and excluded the children who have taken medicines such as antihistamines or other drugs which could affect the result of skin prick test. Finally, a total of 567 children who have tested were enrolled in this study.

2.2. Assessments

The presence of allergic symptoms was determined using the following question in the questionnaire: “In the past 12 months, has your child ever had a problem with sneezing, or a runny, or a blocked nose when he/she DID NOT have a cold or the flu?” [8]. For each subject, 4 mL of blood sample was collected. Of the 4 mL of the blood sample, 1 mL was transferred to an ethylenediaminetetraacetic acid tube for the estimation of leukocyte count, and 1.5 mL was transferred to a cryotube for the measurement of ECP and TlgE concentrations. Eosinophils and leukocytes were counted using XE-2100D (Sysmex Corp., Japan). Direct current detection technique was used to determine eosinophil count, and laser flow cytometry was used to determine leukocyte count. ECP was measured using enzyme-linked immunosorbent assay according to the manufacturer’s instructions (Aviscera Bioscience, Inc., CA, USA). TlgE concentration was determined using an immunoradiometric assay performed using 1470 WIZARD (PerkinElmer Corp., Finland). An SPT was performed for 26 common aeroallergens in Korea (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat, dog, *Cladosporium*, *Alternaria*, timothy grass, mugwort, barley, rape-

seed, Japanese hop, ryegrass, hen’s egg (white), tuna, cow milk, soya flour, mussel, yolk, shrimp, chicken meat, pork, peach, peanut, wheat flour, citrus red mite, and Japanese cedar). Histamine (1 mg/mL) was used as the positive control. Normal saline mixed with 50% glycerin was used as the negative control. Skin prick was performed on the inner forearm. The size of each wheal was calculated as the mean of the longest diameter (A) and the diameter perpendicular to the first axis at the midpoint (B), i.e. (A + B)/2. A wheal diameter of >3 mm, or a wheal of size the same as the size of or larger than the positive control indicated a positive reaction.

2.3. Statistical analysis

All the statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software version 17.0 (SPSS Inc., Chicago, IL, USA) and MedCalc version 15.0. A *p* value <0.05 was considered statistically significant. Chi-square test was used. The cut-off value and the usefulness of the serologic markers (SEP, ECP concentration, and TlgE concentration) were determined using the receiver operating characteristic (ROC) curve.

3. Results

At first, we evaluated the development of allergic nasal symptoms 2 years after the initial examination. In total, 313 children who did not show symptoms initially, regardless of allergic sensitization, were included. Of the 3 serologic markers, SEP was associated with newly developed allergic nasal symptoms, and its cut-off value was 3.8 (Fig. 1). The other 2 serologic markers did not show significant association with newly developed allergic nasal symptoms. Of 231 children with SEP below 3.8 initially, only 13.9% (*n* = 32) developed allergic nasal symptoms 2 years later. However, of 82 children with SEP above 3.8, 28% (*n* = 23) developed allergic nasal symptoms. These results suggest that SEP was associated with newly developed allergic nasal symptoms (*p* = 0.004) (Table 1). On the ROC curve, the area under the curve (AUC) of SEP (0.596) was greater than the AUC of the other 2 serologic markers.

Next, the development of allergic sensitization 2 years later was assessed. One hundred ninety-one children who initially showed negative results on the SPT, regardless of the presence of allergic nasal symptoms, were enrolled. Forty-two children showed allergic nasal symptoms and 149 children did not. TlgE concentration was associated with newly developed allergic sensitization, and its

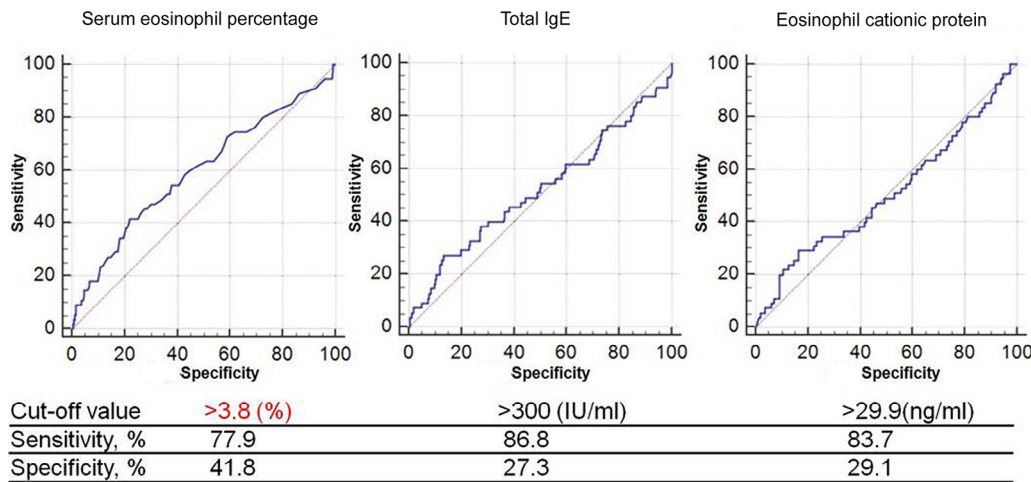


Fig. 1. Receiver operating characteristic (ROC) curve for allergic symptoms that developed 2 years later in 313 subjects without allergic symptoms at the initial examination. Among the 3 serologic markers, serum eosinophil percentage was associated with newly developed allergic symptoms. The cut-off value of SEP was 3.8 (Odds ratio = 2.424, *p* = 0.006).

Table 1

Changes in allergic nasal symptoms according to serum eosinophil percentage (SEP) in 313 children without allergic nasal symptoms at the initial examination, regardless of the presence of allergic sensitization ($p = 0.004$).

	SEP <3.8%	SEP ≥3.8%
Without development of allergic nasal symptoms (n = 258)	199 (86.1%)	59 (72%)
Newly developed allergic nasal symptoms (n = 55)	32 (13.9%)	23 (28%)
Total (n = 313)	231	82

cut-off value was 17.7 IU/mL (Fig. 2). The other 2 serologic markers did not show significant association with newly developed allergic sensitization. Among 66 children with the initial TlgE concentration below 17.7 IU/mL, only 15% (n = 10) developed allergic sensitization 2 years later. On the other hand, among 125 children with the initial TlgE concentration above 17.7 IU/mL, 47% (n = 58) developed allergic sensitization ($p < 0.001$) (Table 2). A pairwise comparison showed that TlgE concentration was the most statistically significant marker for the prediction of allergic sensitization. The AUC of TlgE concentration (0.688) was greater than that of the other 2 serologic markers.

Finally, we investigated the results of 42 children who showed allergic symptoms but who initially showed negative results on the SPT. TlgE concentration was significantly associated with newly developed allergic sensitization. The cut-off value of TlgE concentration was 17.4 IU/mL (Fig. 3). Of 28 children with the initial TlgE concentration above 17.4 IU/mL, 68% (n = 19) showed newly developed allergic sensitization 2 years later, whereas none of the children with TlgE concentration below 17.4 IU/mL (n = 14) developed allergic sensitization. These results suggest that the concentration of TlgE was strongly associated with newly developed allergic sensitization ($p < 0.001$) (Table 3). The AUC of TlgE concentration (0.874) was much greater than that of the other serologic markers.

4. Discussion

The incidence of AR has been on a steady rise. Especially, AR prevalence in adults is 10–30%, whereas that in children is up to 40% [1]. Moreover, 40% of AR patients develop symptoms of AR before 6 years of age [2]. AR considerably affects the quality of life and has remarkable repercussion if left untreated [9]. AR is associated with asthma, sinusitis, chronic middle-ear effusion, lymphoid hypertrophy, obstructive sleep apnea, and consequent disorders of behavior

Table 2

Changes in allergic sensitization according to total immunoglobulin E (TlgE) concentration in 191 children with negative skin prick test (SPT) results at the initial examination, regardless of the presence of allergic nasal symptoms ($p < 0.001$).

	TlgE concentration <17.7 IU/mL	TlgE concentration ≥17.7 IU/mL
Negative SPT results in both 2012 and 2014 (n = 123)	56 (85%)	67 (53%)
Newly positive results on the SPT (n = 68)	10 (15%)	58 (47%)
Total (n = 191)	66	125

and learning performance [3,9]. Despite its high prevalence and associated disorders, pediatric AR could be often underdiagnosed or misdiagnosed as other disorders such as recurrent upper respiratory infections [2,3]. Various tests including SPT have been used to diagnose AR. However, the results of allergic tests could alter with time [6,7]. Newly developed sensitization could occur, and negative conversion of allergic test was also possible. Therefore, a series of tests are needed in cases where allergic sensitization is suspected.

An SPT and various in vivo tests that measure the concentrations of specific IgE were used to confirm allergic sensitization. In addition, TlgE concentration, SEP, and ECP concentration could be determined for diagnostic or research purpose, and the concentrations of these markers usually increase in persons with allergic sensitization or allergic disease. SEP is considerably higher in children with AR than in healthy controls [10]. Seasonal increases in SEP occur in AR patients [11], and SEP is related to the severity of symptoms in seasonal AR [10–12]. In a randomized population-based study with 2167 participants, SEP was related to nasal symptoms [13]. Many studies showed the relationship between serum ECP concentration and AR. Serum ECP concentration is remarkably higher in children with AR than in healthy controls, and it is positively related to the disease activity and symptom severity [10,11,14]. Although the concentration of TlgE can also increase in non-allergic diseases such as infectious disease and neoplastic disease, measurement of TlgE is a cost-effective method for the diagnosis of allergic disease. In a randomized population-based study with 1512 adults, subjects with high concentrations of TlgE were 37 times more likely to show IgE antibodies specific for ≥1 common allergen [15]. Furthermore, TlgE concentration has a high negative predictive value, indicating that a specific IgE test is unnecessary in patients with low TlgE concentration (<10 IU/mL) [16]. In a

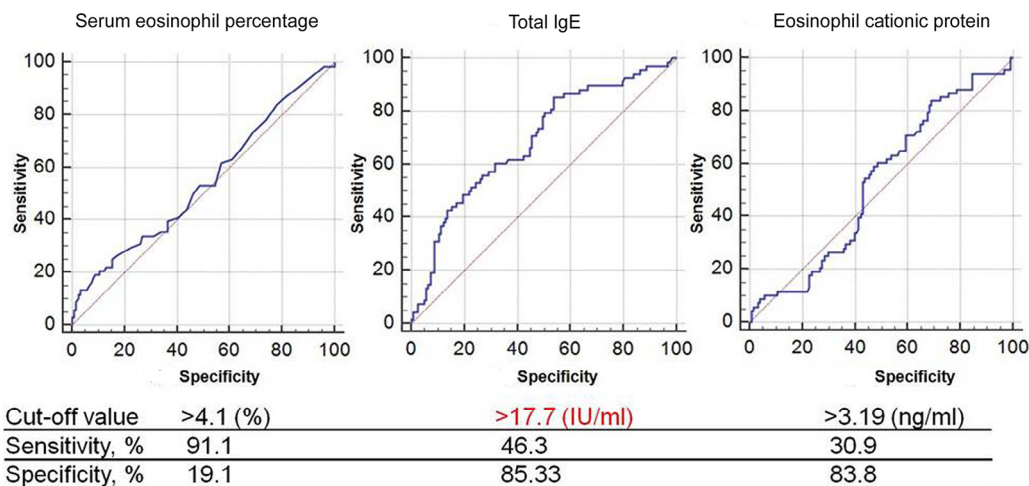


Fig. 2. ROC curve for allergic sensitization that developed 2 years later in 191 subjects without allergic sensitization at the initial examination. Among the 3 serologic markers, TlgE was associated with newly developed allergic sensitization. The cut-off value of TlgE was 17.7 (Odds ratio = 4.848, $p < 0.001$).

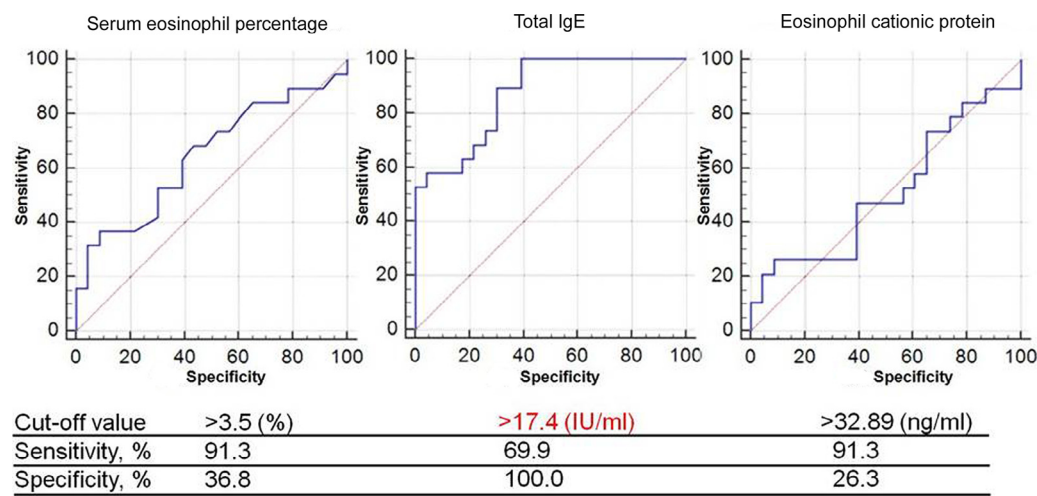


Fig. 3. ROC curve for allergic sensitization that developed 2 years later in 42 subjects who showed allergic symptoms but negative skin prick test (SPT) results at the initial examination. Among the 3 serologic markers, TIgE was strongly associated with newly developed allergic sensitization. The cut-off value of TIgE was 17.4, which was similar to the value presented in Fig. 2.

longitudinal observational study in children with high TIgE concentrations, TIgE concentration was a useful predictive parameter for polysymptomatic allergy [6]. In a previous study on the predictive capabilities of allergy markers in AR, serum TIgE concentration, serum ECP concentration, and blood eosinophil percentage were found to be strong predictors of AR in a clinical setting [4]. However, a patient with negative results on allergic tests may have increased IgE concentration, ECP, and SEP concentration. Furthermore, whether the elevated TIgE concentration, ECP, and SEP concentration were associated with future risk for allergic sensitization or disease remains unknown.

In the present study, the cut-off value of 3.8% for SEP showed that SEP is a good predictor of the development of allergic symptoms. Moreover, the cut-off value of 17.7 IU/mL for TIgE might predict newly developed allergic sensitization in the 2 years of follow-up. When we applied the same tests for children with allergic nasal symptoms but who did not show allergic sensitization, we obtained similar results. A high TIgE concentration (≥ 17.4 IU/mL) might be a useful marker for newly developed allergic sensitization. This result corresponds to that of the previous study that showed that TIgE concentration was an important predictor of alterations in the SPT results [7]. Furthermore, our analysis showed that TIgE concentrations measured in 2012 were highly correlated to those measured in 2014 (Spearman's rank correlation coefficient = 0.882, $p < 0.001$; Supplemental Fig. S1). In addition, only 10% of children with TIgE above 17.4 in 2012 showed TIgE level below 17.4 in 2014. On the contrary, 25% of children with TIgE below 17.4 in 2012 showed the TIgE level above 17.4 in 2014. We postulated that the risk of new allergic sensitization is low in children with negative SPT results and TIgE concentration < 17.4 IU/mL. However, our results suggest that the incidence of newly developed allergic sensitiza-

tion was high in children with negative SPT results and TIgE concentration > 17.4 IU/mL, although the results are not conclusive. Furthermore, a series of tests for allergic sensitization were needed for those children. On the other hand, additional tests for allergic sensitization may not be needed for children with negative SPT results and TIgE concentration < 17.4 IU/mL.

Our study has several limitations. First, SPTs, though widely used to confirm allergic sensitization in various allergic diseases, may yield false-positive and false-negative results. We considered the SPT the standard test for allergic sensitization; therefore, the possibility of false-positive and false-negative results could affect the results of our study. Second, we did not measure the serum concentration of specific IgE. Many previous studies showed that serum TIgE concentration could be a valuable marker for the prediction of serum specific IgE sensitization [4,6,15,16]. Therefore, further studies on the association between TIgE concentration and alteration in specific IgE could be interesting. Third, TIgE concentration, ECP concentration, and SEP could increase in other conditions, including parasite infections, fungal infections, inflammatory diseases (e.g., Kimura disease and Churg–Strauss vasculitis), and hematologic malignancies (e.g., Hodgkin lymphoma and leukemia) [17,18]. These factors were not considered in this analysis. Therefore, other conditions that could affect TIgE concentration, ECP concentration, and SEP should be considered. Finally, the AUCs of SEP (0.596) for newly developed allergic nasal symptoms and TIgE (0.688) for newly developed allergic sensitization were not so high, even though the p values for AUCs were statistically significant (0.006 and < 0.001 , respectively). But, we could not explain the clinical importance of this low AUC value, and not understand the clinical significance exactly. However, the strength of our study is that it was a longitudinal study conducted in children of a specific age group.

In conclusion, the present study determined the association of serologic markers with allergic symptoms and sensitization for the prediction of AR. Serum eosinophilia and TIgE concentration may be useful predictors of allergic symptoms and allergic sensitization, respectively. Further study is needed to determine the predictive ability of serum eosinophilia and TIgE for future risk of an allergy.

Author contributions

J.W.K. and J.H.K. designed the research; K.W.L., S.C.H., H.S.L., and J.H.K. analyzed the data; J.W.K. and S.C.P. wrote the paper. J.W.K.,

Table 3
Changes in allergic sensitization according to TIgE concentration in 42 children with negative SPT results and allergic nasal symptoms on initial examination ($p < 0.001$).

	TIgE concentration <17.4 IU/mL	TIgE concentration ≥17.4 IU/mL
Negative SPT results in both 2012 and 2014 (n = 23)	14 (100%)	9 (32%)
Newly positive results on SPT (n = 19)	0 (0%)	19 (68%)
Total (n = 42)	14	28

J.H.K., and S.C.P. revised the manuscript. J.W.K. had primary responsibility for final content. All authors read and approved the final manuscript.

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Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ijporl.2016.05.005>.

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