

Original article

Cc-chemokine eotaxin as a marker of efficacy of specific immunotherapy in patients with intermittent IgE-mediated allergic rhinoconjunctivitis

Background: Allergen-specific immunotherapy (SIT) is believed to be a valuable remedy in several allergic diseases; however, an accurate immunological marker of the efficacy of this treatment method has not been found yet. Cc-chemokine eotaxin, owing to its selective action on eosinophils, seems to play an important role in the pathophysiology of allergic response. The purpose of this study was to assess the usefulness of eotaxin in monitoring of SIT efficacy in patients with IgE-mediated allergic rhinoconjunctivitis.

Methods: One hundred and twenty-two patients with seasonal IgE-mediated allergic rhinoconjunctivitis due to tree- (birch, $n = 42$; hazel/alder, $n = 14$) or grass/cereal- ($n = 66$) pollen received allergen-specific immunotherapy. Serum eotaxin levels were determined four times in every patient, shortly before immunotherapy (Evaluation 0), immediately after the treatment (Evaluation 1), in the height of pollen season (Evaluation 2) and at least 2 weeks after the pollen season (Evaluation 3). Serum eotaxin levels were simultaneously measured in 59 healthy people from the control group. Changes in serum eotaxin levels were assessed in the healthy and allergic groups. Clinical symptoms of IgE-mediated rhinoconjunctivitis were evaluated and compared with serum eotaxin concentration changes.

Results: Mean values of eotaxin concentrations in serum during Evaluations 0–3 did not significantly differ in the healthy subjects and the patients with IgE-mediated rhinoconjunctivitis ($P > 0.05$). Moreover, no statistically significant differences in the serum eotaxin levels between the visits were observed in the patients who received immunotherapy ($P > 0.05$); however, immediately after immunotherapy (Evaluation 1) the mean serum level of eotaxin was lowest and closest to the serum eotaxin concentration in the control group at the same visit. No significant correlation between the mean value of the serum eotaxin level in the height of pollen season ($r = -0.12$, $P > 0.05$) or mean changes of the eotaxin levels between Evaluations 2 and 1 ($r = -0.03$, $P > 0.05$), on the one hand, and the cumulative score of symptoms and drug, on the other, were found.

Conclusions: The results allow to conclude that although eotaxin plays a significant role in the mechanism of antigen-specific immunotherapy, its serum expression remains a poor marker of SIT efficacy.

**K. Jahnz-Różyk, T. Targowski,
E. Głodzinska-Wyszogrodzka,
T. Płusa**

Department of Internal Medicine, Pneumology
and Allergology, Central Hospital of Military School
of Medicine, Warsaw, Poland

Key words: allergen-specific immunotherapy; eotaxin;
IgE-mediated allergic rhinoconjunctivitis.

K. Jahnz-Różyk
Department of Internal Medicine, Pneumology
and Allergology
Central Hospital of Military School of Medicine
Warsaw
Poland

Accepted for publication 14 November 2002

Specific immunotherapy (SIT) using allergen extracts is highly effective in seasonal rhinoconjunctivitis and it is the only treatment that has been shown to confer a long-term benefit up to several years following discontinuation of the treatment (1). Many compounds and cells are involved in allergic response, but search for an immunological marker of SIT effectiveness remains still unsatisfactory. Eotaxin is a unique selective chemokine, which has been implicated in the pathogenesis of allergic inflammation (2–4). In numerous studies, correlation

between an elevated concentration of eotaxin and allergen exposure has been confirmed (5, 6). Owing to its unusual selective influence on migration of eosinophils, eotaxin seems to be a promising marker of allergic inflammation. In this study, we made an attempt at assessment of the usefulness of serum eotaxin measurements in monitoring of the efficacy of allergen-specific immunotherapy in patients with sensitization to the most common plant aeroallergens in Central Europe.

Materials and methods

One hundred and twenty-two non-smoking patients with seasonal IgE-mediated allergic rhinoconjunctivitis due to tree- (birch, hazel, alder) or grass/cereal-pollen were recruited from outpatients of the Department of Internal Diseases, Allergology and Pneumology of Military School of Medicine, Warsaw, Poland (7). All subjects had at least a 2-year positive history of rhinoconjunctivitis (42 of them had mild seasonal bronchial asthma additionally), confirmed by positive skin prick tests (class ++ or more, Allergopharma, Germany) and significant level of specific IgE (class II or higher, FAST Pharmacia CAP) (Table 1). None of the patients had symptoms beyond the pollen season and none had received immunotherapy or immunosuppressive treatment previously. None of them had suffered from immunodeficiencies, malignancies, psychiatric or infectious diseases. The recruitment was conducted before the pollen season.

The patients received a standardized preseasonal SIT (Allergovit[®], Allergopharma, Germany), depending on clinical data. Allergovit[®] has been granted the certificate of GMP (Good Manufacture Practice). Allergovit[®] contains allergoids of plant pollen, which are repeatedly dialyzed, adsorbed to aluminum hydroxide, suspended in physiological saline, reserved with 0.4% phenol and standardized in therapeutic units (TU). Allergoids are prepared in two 3-ml vials. Vini A contains 1000 TU/ml and vini B contains 10 000 TU/ml. The standardized extracts contain appropriate allergoid (birch 100%, grass/cereals 100% or hazel 50% and alder 50%).

The administration schedule involved increasing doses of allergoid suspension (0.1, 0.2, 0.4, 0.8 ml from vini A and 0.15, 0.30, 0.60 ml from vini B) to be injected every 7–14 days. The total amount of the extract administered was 12 000 TU. Immunotherapy was terminated at least 2 weeks before the pollen season.

During the study, the patients could use, if needed, loratadine (one tablet of 10 mg per day), intranasal (200 µg per day) and/or inhaled (400 µg per day) budesonide and fenoterol (400 µg per day).

Serum eotaxin levels were measured shortly before immunotherapy (Evaluation 0), after the treatment immediately (Evaluation 1), in the height of the pollen season (Evaluation 2) and at least 2 weeks after the pollen period (Evaluation 3) – according to the pollen season for birch, hazel/alder or grass/cereals in Warsaw, Poland, in 2000. The serum eotaxin levels were simultaneously measured in 59 healthy people from the control group. All subjects from both groups were non-smoking and did not have any infection during 3 months preceding the study. The subjects from the control group had a negative family history of allergic diseases and had negative results of allergic skin prick tests (Allergopharma, Germany).

Table 1. Clinical data of subjects

	All	Female	Male
<i>Study group</i>			
Number of subjects (n)	122	44	78
Mean age (years) ± SD	30.0 ± 10.6	30.8 ± 10.8	29.6 ± 10.6
<i>Sensitization</i>			
Trees (n)	56	18 (40.9%)	38 (48.7%)
Birch	42	12 (28.6%)	30 (71.4%)
Hazel, alder	14	6 (42.9%)	8 (57.1%)
Grass/cereals (n)	66	26 (59.1%)	40 (51.3%)
<i>Control group</i>			
Number of subjects	59	41	18
Mean age (years) ± SD	29.7 ± 7.6	29.7 ± 6.7	29.5 ± 9.4

The quantitative ELISA method (R & D kits, USA) was used to determine the serum eotaxin levels (the detection threshold of the method is 5.0 pg/ml) (8).

During the third visit (Evaluation 2), all patients were asked to assess themselves if the symptoms of the disease: had improved, had not changed or had deteriorated in comparison to the previous pollen season. Moreover, each patient was asked to record in a card the intensity of allergic symptoms such as: sneezing, nasal obstruction, rhinorrhea, nasal/conjunctival itching, lacrimation, cough and wheezing. Each symptom was graded on a subjective scale as follows: 0 – no symptom, 1 – mild, 2 – moderate, 3 – severe. Moreover, each dose of each symptomatic drug taken was recorded as score 1. A mean cumulative clinical symptoms and drug intake score was calculated for every patient by addition of all scores of symptoms and taken medicines (9, 10).

After the assessment of normality of distribution, quantitative data were analyzed by the ANOVA test and *t*-test for paired and nonpaired parameters. For *post hoc* comparison the Tukey test was used. Kruskal–Wallis and Mann–Whitney tests were used in the assessment of nonpaired, nonnormally distributed variables. The relationship between the numerical parameters was studied by the Pearson correlation coefficient (*r*) test. *P* of less than 0.05 was considered significant.

The study protocol was approved by the ethics committee of the Military University School of Medicine in Warsaw (Poland) and each patient signed an informed consent form before entering the trial.

Results

No one dropped out from the study groups. No subject receiving SIT experienced systemic adverse events. The mean values of eotaxin concentrations in serum during Evaluations 0–3 did not significantly differ in the healthy subjects and the patients with IgE-mediated rhinoconjunctivitis. Moreover, no statistically significant differences in the serum eotaxin levels between the visits were observed in the patients treated with immunotherapy (*P* = 0.24); however, immediately after immunotherapy (Evaluation 1) the mean serum level of eotaxin was lowest and most nearing the serum eotaxin concentration in the control group at the same visit. The mean values of eotaxin are presented in Table 2.

After immunotherapy, an evident decrease of the serum eotaxin level was observed in the patients sensitized to birch only (Evaluation 1 vs Evaluation 0), while desensitization had no statistically significant influence on decrease of the serum concentration of eotaxin in the patients sensitized to grass/cereals or hazel/alder. During the pollen season (Evaluation 2), the decline tendency was still present in case of the patients sensitized to birch. The patients sensitized to hazel/alder had a perceptibly increased mean serum level of eotaxin; however, a low numerical force of this group probably resulted in an insignificant value of *P*-coefficient. In the patients sensitized to grass/cereals changes in the serum eotaxin level were slight.

The differences between the mean serum eotaxin levels in the subjects sensitized to trees and grass/cereals at the consecutive visits are presented in Table 2.

Table 2. Mean values of serum eotaxin levels in study and control groups during each evaluation

Mean serum eotaxin level (pg/ml)	Evaluation 0, mean (SD)	Evaluation 1, mean (SD)	Evaluation 2, mean (SD)	Evaluation 3, mean (SD)	<i>P</i> value
Study group (<i>n</i> = 122)	116.3 (48.8)	105.7 (42.7)	110.2 (44.6)	106.8 (41.7)	<i>P</i> = 0.2
Birch (<i>n</i> = 42)	119.1 (SD 45.5)	95.2* (SD 45.7)	92.2** (SD 42.2)	110.5 (SD 36.1)	* <i>P</i> = 0.04, ** <i>P</i> = 0.02
Hazel/alder (<i>n</i> = 14)	113.5 (SD 48.2)	106.4 (SD 43.9)	144.2 (SD 63.5)	121.1 (SD 47.6)	<i>P</i> = 0.25
Grass/cereals (<i>n</i> = 66)	115.1 (SD 51.6)	111.6 (SD 40.0)	114.5 (SD 36.1)	101.4 (SD 43.3)	<i>P</i> = 0.24
Control group (<i>n</i> = 59)	103.1 (53.2)	104.0 (52.1)	109.3 (52.0)	100.7 (50.2)	<i>P</i> = 0.8
<i>P</i> value study vs control group	<i>P</i> = 0.1	<i>P</i> = 0.8	<i>P</i> = 0.9	<i>P</i> = 0.4	

Table 3. Differences in mean variation (Δ) of eotaxin value between Evaluations 1 and 0 ($E1 - E0$), Evaluations 2 and 1 ($E2 - E1$), Evaluations 3 and 2 ($E3 - E2$), and Evaluations 3 and 0 ($E3 - E0$) in patients sensitized to aeroallergens and healthy people (*n* = number of sensitized patients)

Mean Δ (pg/ml) of eotaxin level between evaluations (E)	Δ ($E1 - E0$)	Δ ($E2 - E1$)	Δ ($E3 - E2$)	Δ ($E3 - E0$)
Study group 0 (<i>n</i> = 122)	-9.1 (SD 40.5)	3.7 (SD 38.7)	-2.9 (SD 45.7)	-6.5 (SD 50.4)
Birch 1 (<i>n</i> = 42)	-23.0 (SD 34.3)	-3.9 (SD 33.2)	18.1 (SD 45.4)	-4.6 (SD 51.9)
Hazel/alder 2 (<i>n</i> = 14)	-7.1 (SD 53.9)	37.7 (SD 44.0)	-16.3 (SD 47.5)	7.8 (SD 64.4)
Grass/cereals 3 (<i>n</i> = 66)	-0.7 (SD 39.2)	1.4 (SD 37.6)	-13.4 (SD 41.2)	-10.7 (SD 46.2)
Control group 4 (<i>n</i> = 59)	0.9 (SD 10.8)	5.2 (SD 11.4)	-8.6 (SD 16.7)	-2.4 (SD 12.7)
<i>P</i> value	1 vs 4 (<i>P</i> = 0.000002); 1 vs 3 (<i>P</i> = 0.01)	2 vs 4 (<i>P</i> = 0.00003); 2 vs 1 (<i>P</i> = 0.01); 2 vs 3 (<i>P</i> = 0.03)	1 vs 2 (<i>P</i> = 0.03); 1 vs 3 (<i>P</i> = 0.001); 1 vs 4 (<i>P</i> = 0.00007)	<i>P</i> = n.s.

Our previous findings had revealed that the serum eotaxin level significantly and positively correlates with age in healthy people and additionally correlates with gender in patients with allergic diseases (11). Thus, in order to eliminate statistical errors resulting from differences between the groups concerning mean age and the number of male and female subjects, mean variations (Δ) of eotaxin value between Evaluations 1 and 0, Evaluations 2 and 1, Evaluations 3 and 2, and Evaluations 3 and 0 were used in comparative analysis of the influence of SIT on the serum eotaxin level in patients sensitized to different aeroallergens.

After immunotherapy, a mean decrease in the serum concentration of eotaxin in the patients with allergy to birch was significantly greater than that in the patients sensitized to grass and/or cereals (*P* = 0.01) and also strongly different in comparison to the values in the control group ($\Delta E1 - E0$ = 0.9 pg/ml) (*P* = 0.00002). In the subjects sensitized to hazel/alder, a significant increase of the mean value of eotaxin concentration between Evaluations 2 and 1 ($\Delta E1 - E0$ = 37.7 pg/ml) was observed in comparison to the control group ($\Delta E1 - E0$ = 5.2 pg/ml) (*P* = 0.000003). Surprisingly, after the pollen season there was a noticeable statistically significant increase of the mean value of the serum eotaxin concentration in birch-sensitized patients only ($\Delta E3 - E2$ = 18.1 pg/ml) in comparison to the rest of the subjects (*P* < 0.05). Finally, no significant difference in the mean change of the eotaxin level between the last and initial visits ($\Delta E3 - E0$) was observed between the groups.

For the assessment of the relationship between the serum eotaxin levels and the scores of clinical symptoms

plus drugs, the mean cumulative scores from the whole day of Evaluation 2 were used.

The mean cumulative score of clinical symptoms and drugs intake in the immunotherapy group was 7.4 points (SD = 5.0; median = 6.0). In spite of differences in individual variations of the serum eotaxin level, no significant differences in the values of symptoms and drugs score between the patients sensitized to birch [median = 7.0, mean (SD) = 7.9 (5.4)], hazel/alder [median = 8.0, mean (SD) = 8.9 (6.0)] and grass/cereals [median = 6.0, mean (SD) = 6.8 (4.5)] (*P* > 0.05) were found. The mean variations (Δ) of eotaxin values between the visits in the healthy and allergic groups are presented in Table 3.

After immunotherapy, during the pollen season 85 (70%) patients declared improvement of clinical symptoms, 30 (24.5%) did not feel any change in the intensity of clinical symptoms and 7 (5.5%) recorded deterioration in comparison to the previous pollen season. The symptom plus drug intake scores of the subgroup with clinical improvement [median = 5, mean (SD) = 5.6 (3.7)] were significantly lower (*P* < 0.05) in comparison to the subgroups with no change of symptoms [median = 10.0, mean (SD) = 11.0 (5.5)] or clinical deterioration [median = 12.0, mean (SD) = 13.4 (4.0)]. Distribution of the scores and individual changes in the serum eotaxin levels between the peak of the pollen season and immediately after immunotherapy ($\Delta E2 - E1$) are presented in Fig. 1.

Unfortunately no significant correlation between the mean value of the serum eotaxin level during the third visit [$E2$: mean (SD) = 110.2 pg/ml (44.6)] or mean

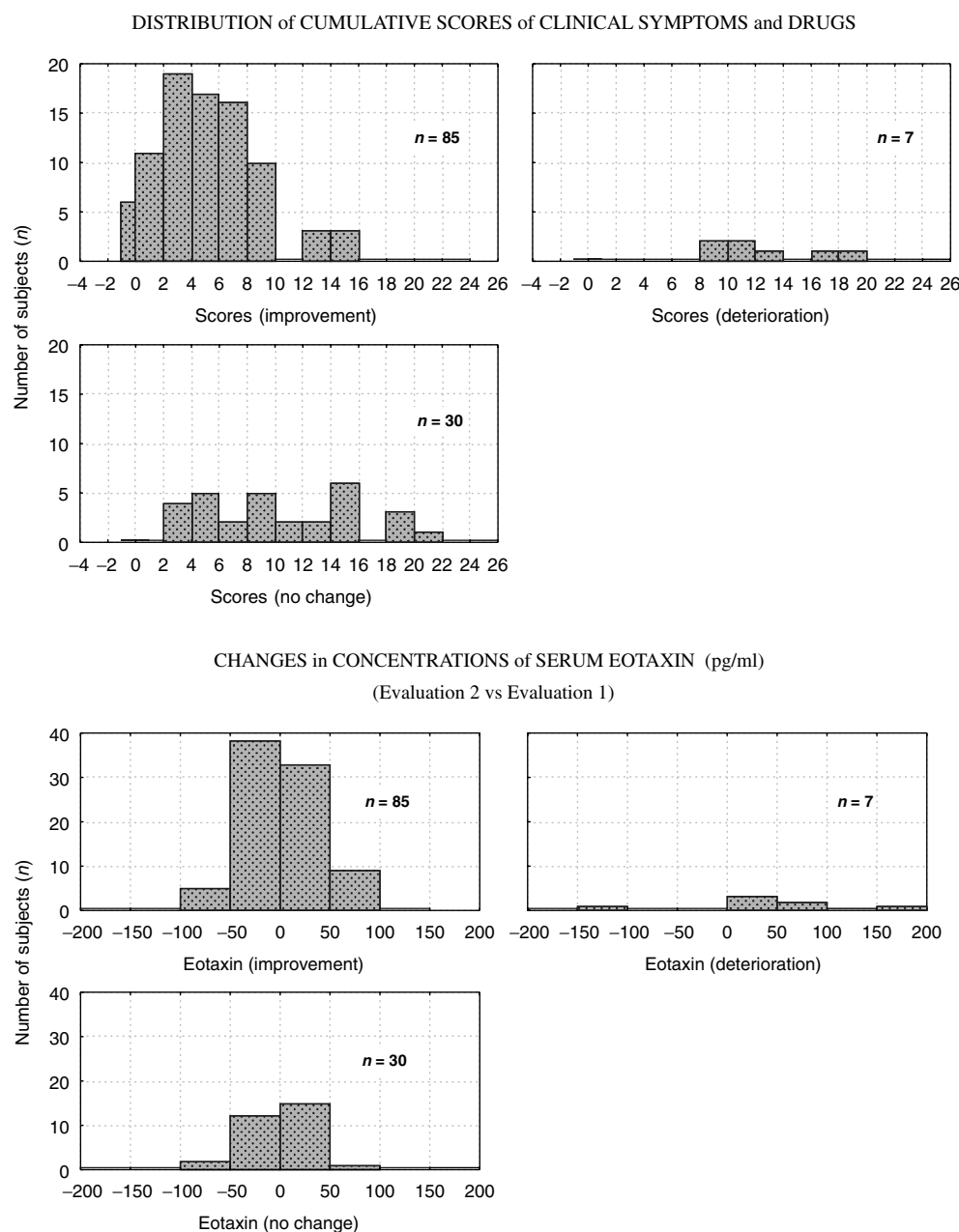


Figure 1. Cumulative scores of clinical symptoms and drugs, and changes in serum eotaxin concentrations in patients who declared clinical improvement, deterioration or no change of symptoms.

individual changes of the eotaxin value between Evaluations 2 and 1 [$\Delta E2 - E1$: mean (SD) = 4.6 pg/ml (38.7)] on the one hand, and the cumulative score of symptoms and drug, on the other, were found [$r = -0.11$ and $r = -0.01$, respectively ($P > 0.05$)].

During the pollen season in the subgroup of patients declaring clinical improvement, 51 subjects did not require any symptomatic drugs, three subjects required intranasal or inhaled drugs only and 31 subjects had to use systemic H_1 -antihistamines (loratadine) additionally. It was found that the mean serum eotaxin levels

significantly decreased between Evaluations 2 and 1 in the 31-patient group taking both systemic and inhaled/intranasal medicines [$\Delta E2 - E1$: mean (SD) = -9.5 pg/ml (39.0), $n = 31$] in comparison to the 51-subject group without any systemic or topical treatment [$\Delta E2 - E1$: mean (SD) = 8.6 pg/ml (29.7)] ($P = 0.02$). No similar relationship between the patients taking inhaled or intranasal drugs only [$\Delta E2 - E1$: mean (SD) = 17.9 pg/ml (39.0)] and those without any treatment [$\Delta E2 - E1$: mean (SD) = 8.6 pg/ml (29.7)] ($P = 0.6$) was observed (Fig. 2). However, because of a very low numerical force

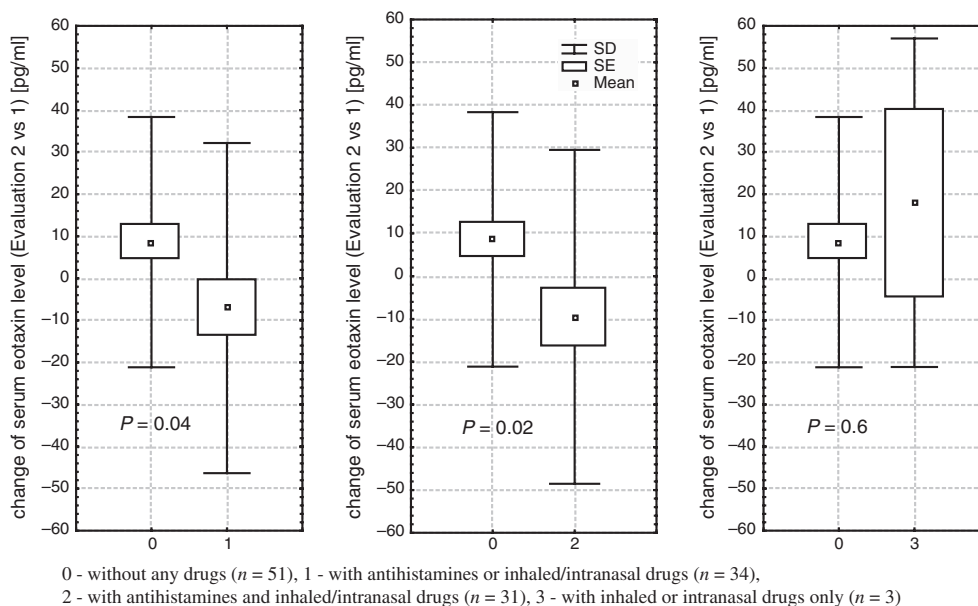


Figure 2. Mean change of serum eotaxin levels in patients with clinical improvement.

of the subgroup treated with inhaled or intranasal medicines only (three persons), the above-mentioned result is not reliable.

Discussion

In a sensitized individual, exposure to allergen will lead to cross-linking of IgE receptors on mast cells and the release of multiple inflammatory mediators including histamine, prostaglandins and leucotriens, which play an important role in an early-phase allergic reaction. A late inflammatory response, the second important phenomenon that contributes to the allergic process, is characterized by an influx of inflammatory cells such as eosinophils and lymphocytes. As an eosinophil-selective chemoattractant, eotaxin is an important contributor of a late-phase allergic response. Several studies of allergic inflammation in animal models have demonstrated that allergen challenge enhances eotaxin gene and protein expression (12–15). Increased mucosal output of eotaxin was demonstrated after allergen exposure in patients with allergic seasonal rhinitis (16). Allergen challenge also increases eotaxin expression in epithelial and endothelial cells of the wall of segmental bronchus in subjects with allergic asthma (17). An increased expression of eotaxin within the peripheral airways of lungs of asthmatic subjects has been shown, suggesting that this chemokine contributes to the small airways and peripheral lung inflammation in asthma (18). Apart from selective chemotactic activity, eotaxin has a very potent priming effect on reactive oxygen species from eosinophils, which may damage cells or tissue, such as the mucosal or bronchial epithelium (2).

There exist scarce published data concerning variations of eotaxin levels in serum of subjects with allergic diseases. It was documented that circulating levels of eotaxin increase in active atopic dermatitis and acute urticaria, and decrease significantly in case of urticaria after remission of the attack (19). Other findings indicate that the plasma eotaxin concentration is significantly higher in asthmatics seeking emergency treatment for exacerbations of symptoms than in control subjects with stable disease matched for age, ethnicity and sex and led to suppose that plasma eotaxin levels are directly related to asthma severity (20).

In this study, first measurements of serum eotaxin levels (Evaluation 0) in people with allergic seasonal rhinoconjunctivitis were done beyond the pollen season, when all patients were free of clinical symptoms. Presumably for that reason no significant elevation of serum eotaxin was found in this group in comparison to the healthy subjects. The results of consecutive measurements (Evaluations 1–3) of the serum eotaxin levels in the allergic group did not differ significantly in comparison to the analogous results from the control group. In our opinion, it was due to influence of allergen-specific immunotherapy. Well-marked decrease of the serum eotaxin levels immediately after immunotherapy (Evaluation 1, $\Delta E1 - E0$) was observed in the patients sensitized to birch. The birch subgroup was also the only allergic subgroup that noted decline tendency of the serum eotaxin levels in the height of the pollen season (Evaluation 2, $\Delta E2 - E1$). It seems that SIT may influence the serum eotaxin levels, especially in patients sensitized to potent aeroallergens with short and intensive pollen period, such as birch. On the other hand, finding elucidation for the evident rising tendency of the plasma

eotaxin levels in the birch subgroup shortly after the end of the pollen season (Evaluation 3, $\Delta E3 - E2$) is problematic. A potential explanation that it could be caused by a compensatory mechanism due to diminished protective activity of immunotherapy is contradictory to the observed decreasing serum eotaxin levels during Evaluation 3 in the patients sensitized to the rest of aeroallergens. Lack of effect of specific immunotherapy on plasma eotaxin levels in the patients sensitized to alder or hazel in the height of the pollen season remains unclear.

In spite of differences in variations of the serum eotaxin levels in the patients sensitized to birch, hazel/alder or grass/cereals during the study, no statistically significant differences in the mean cumulative clinical symptoms and drugs intake scores between these groups were observed. Moreover, no significant linear correlation between the intensity of symptoms and the serum eotaxin level in the height of pollen season ($E2$) or dynamic changes of the eotaxin concentrations between Evaluations 2 and 1 ($\Delta E2 - E1$) was found. In our opinion, these findings seem to disqualify serum eotaxin as a marker of efficacy of SIT. However, it should be underlined that lack of significant differences in the mean serum eotaxin levels during the pollen period ($E2$) between all patients with rhinoconjunctivitis, receiving SIT and the healthy control group proves that eotaxin contributes to the mechanism of SIT.

Another finding worth attention is the significant decrease of the mean plasma eotaxin level during the pollen season ($\Delta E2 - E1$) in 34 patients declaring clinical improvement after SIT and taking additional symptomatic medicines during the pollen season in comparison to 51 patients who declared clinical improvement and did not receive any symptomatic treatment. After exclusion of three patients, who did not take antihistamines, from the group of 34 additionally treated patients, the difference in mean change of the serum eotaxin levels between the additionally treated and nontreated groups became more perceptible in statistical analysis. We suppose that it

may be caused mainly by anti-inflammatory properties of H_1 -antihistamines; however, it is a very controversial allegation and further studies on that issue are necessary. It has been recognized for some years that a number of the second-generation antihistamines appear to possess some anti-allergic, anti-inflammatory effect that cannot be explained by antagonism of the H_1 -receptor only (21).

One of them, loratadine, has been reported to inhibit chemotaxis of eosinophils *in vitro* (22). In another study, premedication with loratadine was *in vivo* shown to reduce significantly the levels of eosinophil cationic protein, eosinophil peroxidase and histamine in nasal lavages and the number of eosinophils and metachromatic cells in nasal scrapings in patients with allergic rhinitis (23). Although, in the aforementioned study the level of eotaxin in nasal lavages was not assessed, it could be assumed that the eotaxin level declined as the number of eosinophils decreased. The results from both above-mentioned trials differed, however, from those of other challenge studies, where loratadine had no inhibitory effect on elevated eosinophils numbers in nasal lavage fluids of patients with allergic rhinitis (24).

Moreover, it was demonstrated that topical glucocorticosteroid (budesonide) treatment attenuates mucosal output of eotaxin increased due to repeated allergen exposure (16). Thus, we also cannot completely exclude that administration of nasal or inhaled drugs in the patients with clinical improvement caused more perceptible decrease of the serum eotaxin level in comparison with the patients who declared clinical improvement after SIT and did not receive any symptomatic medicines.

Reassuring, the influence of symptomatic anti-allergic medicines on changes in serum eotaxin levels should be elucidated in subsequent studies. Especially trials concerning comparative analysis of the influence of antihistamines on the mucosal and serum eotaxin expression should be undertaken. We conclude that although eotaxin plays an important role in the mechanism of immunotherapy, its serum expression remains a poor marker of efficacy of allergen-specific immunotherapy.

References

1. DURHAM SR, WALKER SM, VARGA EM, et al. Long-term clinical efficacy of grass pollen immunotherapy. *New Engl J Med* 1999;**341**:468–475.
2. HONDA K, CHIHARA J. Eosinophil activation by eotaxin – eotaxin primes the production of reactive oxygen species from eosinophils. *J Allergy Clin Immunol* 1999;**54**:1262–1269.
3. MORITA E, KAMEYOSHI Y, HIRAGUN T, MIHARA T, YAMAMOTO S. The C–C chemokines, RANTES and eotaxin, in atopic dermatitis. *J Allergy Clin Immunol* 2001;**56**:194–195.
4. JAHNZ-RÓZYK K. Eotaxin – marker of allergic inflammation in asthma. *Int Rev Allergol Clin Immunol* 2000;**6**:5–10.
5. YAMADA H, YAMAGUCHI M, NAKAJIMA T, et al. Eotaxin in induced sputum of asthmatics: relationship with eosinophils and eosinophil cationic protein in sputum. *J Allergy Clin Immunol* 2000;**55**:392–397.
6. BROWN JR, KLEIMBERG J, MARINI M, SUN G, BELLINI A, MATTOLI S. Kinetics of eotaxin expression and its relationship to eosinophil accumulation and activation in bronchial biopsies and bronchoalveolar lavage (BAL) of asthmatic patients after allergen inhalation. *Clin Exp Immunol* 1998;**114**:137–146.
7. JOHANSSON SGO, O'B HOURIHANE J, BOUSQUET J, et al. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* 2001;**56**:813–824.

8. MORITA A, SHIMOSAKO K, KIKUOKA S, et al. Development of a sensitive enzyme-linked immunosorbent assay for eotaxin and measurement of its levels in human blood. *J Immunol Methods* 1999;**226**:159–167.
9. GUEZ S, VATRINET C, FADEL R, ANDRE C. House-dust-mite sublingual-swallow immunotherapy (SLIT) in perennial rhinitis: a double-blind, placebo-controlled study. *Allergy* 2000;**55**:369–375.
10. ARIANO R, KROON AM, AUGERI G, CANONICA GW, PASSALACQUA G. Long-term treatment with allergoid immunotherapy to *Parietaria*. Clinical and immunologic effects in randomized, controlled trial. *Allergy* 1999;**54**:313–319.
11. JAHNZ-RÓŻYK K, TARGOWSKI T, GŁODZINSKA-WYSZOGRODZKA E, PŁUSA T. Eotaxin in IgE-mediated rhinoconjunctivitis. *Allergy* 2002;**57**:958–959.
12. JOSE PJ, GRIFFITHS-JOHNSON DA, COLLINS PD, et al. Eotaxin: a potent eosinophil chemoattractant cytokine detected in a guinea-pig model of allergic airways inflammation. *J Exp Med* 1994;**179**:881–887.
13. ROTHENBERG ME, LUSTER AD, LILLY CM, DRAZEN JM, LEDER P. Constitutive and allergen-induced expression of eotaxin mRNA in the guinea-pig lung. *J Exp Med* 1995;**181**:1211–1216.
14. COLLINS PD, MARLEAU S, GRIFFITHS-JOHNSON DA, JOSE PJ, WILLIAMS TJ. Cooperation between interleukin-5 and the chemokine eotaxin to induce eosinophil accumulation in vivo. *J Exp Med* 1995;**182**:1169–1174.
15. FUKUYAMA S, INOUE H, AIZAWA H, et al. Effect of eotaxin and platelet-activating factor on airway inflammation and hyperresponsiveness in guinea pigs in vivo. *Am J Respir Crit Care Med* 2000;**161**:1844–1849.
16. GREIFF L, PETERSON H, MATTSSON E, et al. Mucosal output of eotaxin in allergic rhinitis and its attenuation by topical glucocorticosteroid treatment. *Clin Exp Allergy* 2001;**31**:1321–1327.
17. LILLY CM, NAKAMURA H, BELOSTOTSKY OJ, et al. Eotaxin expression after segmental allergen challenge in subjects with atopic asthma. *Am J Respir Crit Care Med* 2001;**163**:1669–1675.
18. TAHA RM, MINSHAL EM, MIOTTO D, et al. Eotaxin and monocyte chemoattractant protein-4 mRNA expression in small airways of asthmatic and non-asthmatic individuals. *J Allergy Clin Immunol* 1999;**103**:476–483.
19. HOSSNY E, ABOUL-MAGD M, BAKR S. Increased plasma eotaxin in atopic dermatitis and acute urticaria in infants and children. *Allergy* 2001;**56**:996–1002.
20. LILLY CM, WOODRUFF PG, CAMARGO CA, et al. Elevated plasma eotaxin levels in patients with acute asthma. *J Allergy Clin Immunol* 1999;**104**:786–790.
21. WALSH GM. The clinical relevance of the anti-inflammatory properties of antihistamines. *Allergy* 2000;**55**:53–61.
22. EDA R, SUGIYAMA H, HOPP RJ, BEWTRA AK, TOWNLEY RG. Effect of loratadine on human eosinophil function in vitro. *Ann Allergy* 1993;**71**:373–378.
23. CIPRANDI G, PRONZATO C, RICCA V, PASSALACQUA G, DANZING M, CANONICA GW. Loratadine treatment of rhinitis due to pollen allergy reduces epithelial ICAM-1 expression. *Clin Exp Allergy* 1997;**27**:1175–1183.
24. BAROODY FM, LIM M, PROUD D, KAGEY-SOBOTKA A, LICHTENSTEIN LM, NACLERIO RM. Effects of loratadine and terfenadine on the induced nasal allergic reaction. *Arch Otolaryngol Head Neck Surg* 1996;**122**:309–316.