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Basophil histamine release, IgE, eosinophil counts, ECP, and EPX are related to the severity of symptoms in seasonal allergic rhinitis

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Key words: allergic rhinitis; basophil histamine release; eosinophil cationic protein; eosinophil protein X; eosinophils; specific IgE.

Background: Serum specific IgE, basophil histamine release, and blood eosinophil parameters are associated with allergic rhinitis, but investigations of the relationship to the severity of allergic symptoms are few and conflicting. Our study aimed to investigate the seasonal changes in the following laboratory tests: specific IgE, basophil histamine release, eosinophil counts, and serum and plasma eosinophil cationic protein (ECP) and eosinophil protein X (EPX), and to analyze, in detail, the relationship of each individual test to the severity of symptoms in rhinitis patients allergic to both birch and grass pollen.

Methods: The above tests were performed on blood samples obtained from 49 allergic rhinitis patients during the birch-pollen season, during the grass-pollen season, and after the seasons. Symptom-medication diaries were filled in during both pollen seasons. We used partial least square (PLS) analysis to establish an optimal statistical link between the symptom score and medication and the laboratory tests, in an investigator-independent way.

Results: Increases in specific IgE, basophil histamine release, eosinophil counts, serum ECP and EPX, and plasma EPX were observed from the birch-pollen season to the grass-pollen season, followed by a decrease from the grass-pollen season to after the pollen seasons, except for the specific IgE. No seasonal changes in plasma ECP and total IgE were seen. The PLS analysis found a relationship between symptom score and medication and the aggregate laboratory tests (F-test value 40.2, correlation 0.34 for the cumulative relation). However, the variation in laboratory tests could explain only half of the total variation in symptoms and less than a quarter of the total variation in medication. The symptom score and, to a minor

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degree, medication were especially correlated with the basophil histamine-release results, with a decreasing relevance of specific IgE, eosinophil counts, total IgE, serum and plasma EPX, and serum ECP. Plasma ECP was not related to the symptom score and medication.

Conclusions: A significant relationship between the severity of allergic rhinitis and various allergic inflammatory markers was found but could account for only a minor part of the variation in the patients' evaluation of their disease.

Abbreviations: ACRI: mean daily capsules of acrivastine; ANTA: mean daily antazoline-naphazoline eye-drops; CAP: specific IgE measured by Pharmacia CAP System; ECP: eosinophil cationic protein; EOS: eosinophil counts; EPX: eosinophil protein X; HR: histamine release; HR15: lowest concentration of extract giving ≥ 15 ng histamine/ml blood in histamine-release test; LEU: leukocyte counts; MAX: maximum histamine released (ng/ml) at any concentration of extract in histamine-release test; NAL: nasal lavage; PLS: partial least square analysis; PRED: mean daily mg of prednisolone; SYMP: mean daily value of total symptom score.

Seasonal allergic rhinitis, or hay fever, is a prevalent allergic inflammatory disease that seems to be well defined by a coincidental occurrence of pollen exposure and symptoms. Accordingly, symptom scores from patient diaries have been much used in attempts to quantify the severity of the allergic disease. This approach is not without problems, however, and a surrogate marker is needed, since diaries are time-consuming and laborious for the patient. Moreover, symptom scores depend upon medication, and several investigators have combined symptom scores and use of medication into a symptom-medication score. However, the relative weight of symptom scores and of medication has varied from one study to another (1–5). Thus, it would be preferable to use a method of weighting symptom and medication scores that is dependent on the actual data, and not on a somewhat arbitrary weight chosen by the investigator.

The allergic inflammation is initiated by allergen cross-linking of specific IgE antibodies, which are attached to mast cells and basophil leukocytes. During the subsequent late phase, the eosinophil leukocyte is the most characteristic cell of the inflammatory reaction in the nasal mucous membrane (6–14). Analysis of markers of these cornerstones of the allergic inflammation could prove highly relevant as a practical and objective replacement of or supplement to symptom-medication scores, provided that they are well correlated with the patient's symptoms. The

literature gives several examples of seasonal variations in IgE serum levels (15, 16), basophil histamine release (HR) (17), and eosinophil activity (18–21), but no clear pattern has emerged.

Accordingly, our study aimed to determine whether a number of different measurements of allergic inflammatory markers would correlate with the seasonally induced symptoms of patients, and, if so, to identify the analysis having the optimal correlation with symptoms, which would thus be the best suited to supplement/replace symptom scores.

Material and methods

Subjects

Forty-nine patients (25 male, 24 female), median age 26 years (range 18–52) with both birch- and grass-pollen-allergic rhinitis for a median of 16 years (range 5–33), and eight adult nonallergic controls (one male, seven female, median age 34 years, range 24–38) were included in the study. The patients participated in a 3-year immunotherapy study (a baseline year without immunotherapy, followed by 2 years of immunotherapy). This study describes the first year, without immunotherapy, aimed at obtaining pretreatment values. All patients had a positive history of birch- and grass-pollen-allergic rhinoconjunctivitis with symptoms in April and May and June through July, corresponding to the birch- and grass-pollen seasons, respectively. No patients had perennial rhinitis. Fifteen of the patients also suffered from mild seasonal asthma, and of these, 10 patients had mild perennial asthma with occasional symptoms. Nine patients were using inhaled local steroids. All the patients had a positive skin prick test (wheal diameter ≥ 3 mm) with *Betula verrucosa* and *Phleum pratense* (Soluprick, ALK, Hørsholm, Denmark) and a positive serum test for specific IgE class ≥ 2 (Pharmacia CAP System, Pharmacia Diagnostics, Uppsala, Sweden) for both allergens.

The study was approved by the local ethics committee of Copenhagen, and written, informed consent was obtained from all patients before the study commenced.

Blood samples, symptom score, and medication

Blood samples were drawn once in the last week of April (visit 1), in the last week of June (visit 2), and in October, 10 weeks after the grass-pollen season (visit 3). One week before visits 1 and 2, the patients recorded symptom score and

medicine intake once a day, and daily pollen counts were reported from the Danish Aerobiological Group of Copenhagen. The severity of five symptoms (sneezing, rhinorrhea, nasal congestion, itchy nose and/or throat, and itchy eyes) were each scored on an arbitrary scale from 0 to 3 (0=no symptoms, 1=mild, 2=moderate, and 3=severe symptoms). The patients were instructed to use medicine only when needed, and to observe changes in medication requirement. The use of the antihistamine acrivastine (8 mg) (Semprex, Wellcome Foundation, Nærum, Denmark) with a maximum intake of three capsules daily, and the eye-drops antazoline-naphazoline (Antistin-Privin, Ciba-Geigy, Basel, Switzerland), with a maximum dosage of six drops in each eye daily, were permitted. If symptoms were not sufficiently alleviated at the maximum dosage of acrivastine and eye-drops, oral prednisolone 10 mg (Nycomed DAK, Copenhagen, Denmark) was prescribed. If more than 20 mg was needed the same day, the patients were asked to contact the investigator for a 1-week prednisolone treatment. For each patient, mean daily values of the total symptom score (SYMP) and mean daily number of acrivastine (ACRI) capsules, antazoline-naphazoline (ANTA) eye-drops, and doses (mg) of prednisolone (PRED) were calculated.

Eosinophil cationic protein (ECP) and eosinophil protein X (EPX) in serum and plasma

It has previously been shown that the levels of ECP and EPX in serum samples are highly influenced by time- and temperature-dependent spontaneous release during blood clotting. In EDTA-treated blood, no further release is seen *in vitro*. Thus, the plasma values may reflect the *in vivo* concentrations, whereas the serum concentrations probably reflect a combination of the number and activity of the circulating eosinophils (22, 23). We therefore decided to measure both serum and plasma values of ECP and EPX. Plasma and serum for ECP and EPX determinations were prepared from blood samples drawn in vacutainers (Labco, Inc., Bucks, UK) with EDTA or without anticoagulant, and were allowed to clot for 2 h at room temperature. Serum and plasma levels of ECP and EPX were determined by ELISA procedures described in detail elsewhere (23, 24). Briefly, both assays are sandwich type using specific rabbit antibodies to ECP/EPX and the biotin-avidin amplification system. Before measurement, the samples were diluted in sample buffer (PBS, pH 7.4) containing 0.1% Tween 20, 0.1% CTAB [*N*-cetyl-*N,N,N*-trimethyl ammonium bromide], 20 mM EDTA, and 0.2% human serum albumin]. ECP and EPX were determined in ranges of 15–1000 and

60–2000 pg/ml. For ECP, the intra- and interassay coefficients of variation were 6% and 10%, respectively. The inter- and intra-assay coefficients of variation for EPX were less than 10% (23, 24).

Eosinophils and total leukocytes

The counting of eosinophils (EOS) and total leukocytes (LEU) was performed automatically by the Technicon H1TM peroxidase method and measured by a Technicon counter (Technicon Instruments Corp., New York, NY, USA).

Histamine release (HR) from basophils

HR from basophil leukocytes was performed by the glass microfiber method (Reference Laboratory, Copenhagen, Denmark), described in detail elsewhere (25). Briefly, 25 µl heparinized whole blood was incubated in glass fiber-prepared microtiter plates for 60 min at 37°C with 25 µl of allergen or anti-IgE, in six and three concentrations, respectively. The allergens were standardized, and partly purified extracts of *Betula verrucosa* or *Phleum pratense* were diluted in 50% glycerol and delivered in stock solution of 10 000 BU/ml (Soluprick, ALK, Hørsholm, Denmark). On the day of HR testing, allergens were diluted 1:10, 1:35, 1:100, 1:350, 1:1000, and 1:3500 (concentration 1–6) in Pipes-AMC buffer. Anti-IgE (410 000 IU/ml) (Behringwerke, Germany) was used at final concentrations of 5125, 1465, and 420 IU/ml (concentration 1–3). After incubation, the plates were rinsed, and residual protein was enzymatically removed. Histamine was analyzed with a Gilson spectro-photo-fluorometer. The sensitivity of the test was 15 ng histamine/ml. The inter- and intra-assay coefficients of variation for the concentration range between 15 and 150 ng/ml were less than 15%.

Two different calculations were used to describe the histamine released by the basophil leukocytes as thoroughly as possible. The HR was calculated as follows:

- 1) basophil releasability (the maximum histamine (ng/ml) released at any concentration [MAX])
- 2) basophil cell sensitivity (the lowest concentration of allergen or anti-IgE giving ≥ 15 ng/ml of histamine [HR15]).

Releasability reflects the intracellular regulation of HR, whereas cell sensitivity reflects the degree of sensitization (26).

Specific IgE and total IgE measurements

Specific IgE to birch (t3) and timothy grass (g6) were measured by an enzyme fluorescence assay with a solid phase of covalently bound allergen to cellulose (Pharmacia CAP System, Pharmacia Diagnostics, Uppsala, Sweden) (CAP), according to the instructions of the manufacturer. In the Pharmacia CAP System, results are reported in kU/l, with a cutoff value of 0.35 kU/l, and an upper limit of 100. Samples exceeding the upper ranges of assay were retested in 1:4 dilutions.

Total serum IgE was measured by the Microparticle enzyme immunoassay on a IMx reader, according to the manufacturer (Abbot Laboratories, Abbot Park, IL, USA). The sensitivity is 0.1 kU/l.

Statistics

The differences in blood tests between subject groups and visits were performed by the one- or two-way analysis of variance and the Student/Newman Keul's test. The relationship between individual *in vitro* tests and between *in vitro* tests and the weighted symptom-medication score (see below) was described by Pearson's correlation coefficient, and hypotheses of no effects were tested for significance by the *t*-distribution. Values of total and specific IgE were natural log-transformed before analysis to ensure normalized residuals.

The partial least square analysis (27) was used to explore the optimal weighting scheme (weighted symptom-medication score) between the four dependent correlated variables SYMP, ACRI, ANTA, and PRED (Y). The laboratory tests performed on all the allergic subjects during their visits to the clinic represent 14 independent correlated variables (X). The PLS analysis is an extension of the usual factor analysis. The analysis predicts the Y data as well as possible; that is, it makes the residuals of Y as small as possible after subtracting a number of principal components, and at the same time produces a linear relationship between X and Y (27, 28). This multivariable model solves the problem of intercorrelation between the variables. All variables were autoscaled before analysis; that is, converted to zero mean and standard deviation equal to 1.

Results

Symptom score and medication

Two patients were excluded from the study due to missing diaries, leaving 47 patients for calculations. The mean birch-

pollen count was low, with 62 birch-pollen grains/m³ (corresponding to the 25% fractile of the daily count of birch pollen in the peak seasons for the years 1977–89) the week before blood tests at visit 1, as it was in the beginning of the birch-pollen season. The symptom scores and use of medication were correspondingly low. The mean average daily symptom score was 1.77 (SD=1.86, range 0–6.14), the mean average daily acrivastine intake was 0.16 (SD=0.42, range 0–1.71), and, finally, the mean average daily number of eye-drops was 0.24 (SD=0.52, range 0–2.29). No patients used prednisolone during the 2 weeks before the blood samples were taken. The mean grass-pollen count was moderate with 47 pollen grains/m³ (corresponding to the 50% fractile of the daily count of grass pollen in the peak seasons for the years 1977–89). A significant increase from visit 1 to visit 2 was observed in symptom score (visit 2: mean 6.26; SD=3.12; range 1–13.86; $P<0.0001$), daily intake of acrivastine (visit 2: mean 1.36; SD=0.93; range 0–3.14; $P<0.0001$), and antazo-line-naphazoline eye-drop use (mean 1.47; SD=1.8; range 0–7.86; $P<0.0001$). Ten patients used a daily mean of 4.6 mg (SD=5.9, range 0.4–17 mg) prednisolone 1 week before the blood sample in the grass-pollen season (visit 2).

Laboratory tests

No differences were observed between the test results for patients with or without prednisolone medication, or patients with or without asthma (data not shown). We therefore included all the patient results in the calculations.

A significant difference in total IgE was observed between the allergic group and the control group. No seasonal change in total IgE of the allergic patients was observed. An increase was observed in birch- and grass-specific IgE from visit 1 to visit 2 (Fig. 1). There was no change from visit 2 to visit 3. There was a positive correlation between total IgE and grass-specific IgE ($r^2=0.60$, $P<0.0001$), whereas the relationship between total IgE and birch-specific IgE was weak ($r^2=0.11$, $P=0.0012$).

In the HR test, an increased release of histamine was observed from the birch-pollen season to the grass-pollen season, for both birch- and grass-pollen allergens and anti-IgE (Fig. 2). There were minor differences in the response to the anti-IgE, and the birch and grass between releasability (MAX) and cell sensitivity (HR₁₅). The MAX increased from visit 1 to visit 2, and decreased from visit 2 to visit 3, in both anti-IgE, and birch and grass, whereas in HR₁₅, the increase was significant only in anti-IgE and birch, and the decrease from visit 2 to visit 3 was significant only for anti-IgE. No difference was observed between visits 1 and 3, except for

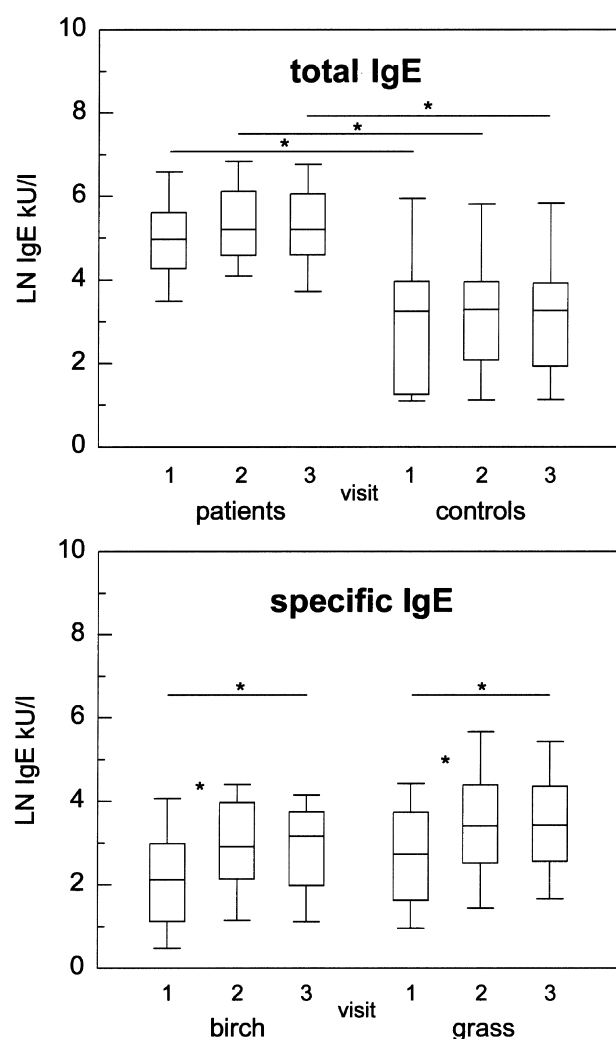


Figure 1. Seasonal changes in blood concentration of total IgE of allergic patients and control subjects, and seasonal changes in birch- and grass-specific IgE (measured by CAP) of allergic patients. Box-and-whisker plot showing 10, 25, 50, 75, and 90% cumulative relative frequencies (centiles). Visit 1) early birch-pollen season; visit 2) mid-grass-pollen season; visit 3) after pollen seasons. * $P < 0.05$.

the birch HR test. A weak but significant correlation was observed between the HR₁₅ and MAX (r^2 between 0.29 and 0.36, $P < 0.0001$). In the birch- and grass-pollen seasons, there was a relationship between the anti-IgE response and the specific birch or grass response in both MAX and HR₁₅ (birch: $r^2 = 0.81$ and 0.47 for MAX and HR₁₅, respectively; grass: $r^2 = 0.81$ and 0.34 for MAX and HR₁₅, respectively; $P < 0.0001$ in all correlations).

A significant increase in EOS, serum ECP, serum EPX, and plasma EPX from the birch-pollen season (visit 1) to the grass-pollen season (visit 2) was observed in the allergic patients, whereas no difference was observed between visit 1 and the period after the pollen seasons (visit 3) (Fig. 3). There was no difference in plasma ECP between visits in the allergic patients, and no difference in all eosinophil

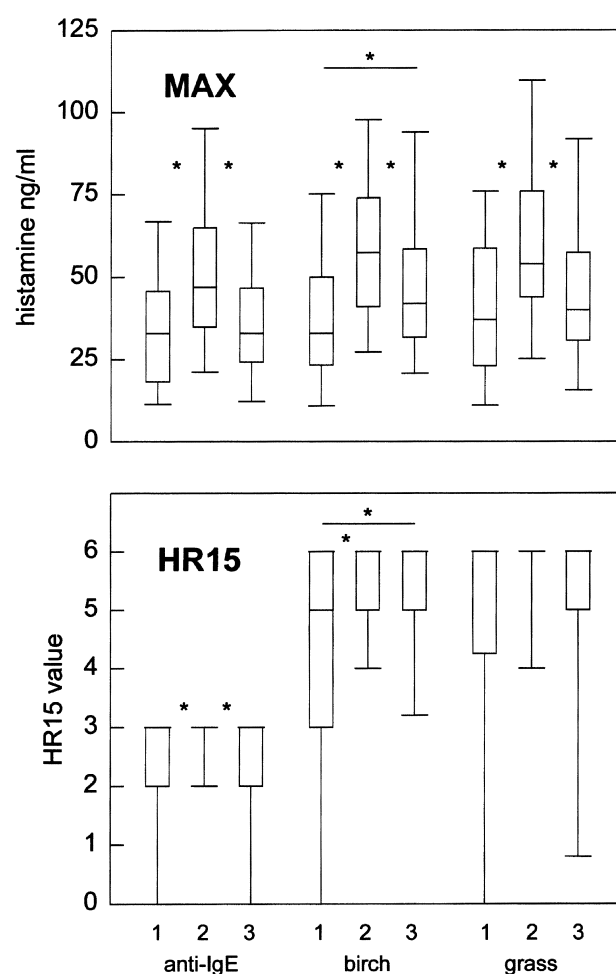


Figure 2. Seasonal changes in basophil histamine release (MAX and HR₁₅) of allergic patients, performed with anti-IgE, and birch- and grass-specific IgE. Box-and-whisker plot showing 10, 25, 50, 75, and 90% cumulative relative frequencies (centiles). HR₁₅ at visit 2 for anti-IgE and grass-specific IgE was maximal at 25% cumulative relative frequency. Visit 1) early birch-pollen season; visit 2) mid-grass-pollen season; visit 3) after pollen seasons. * $P < 0.05$.

parameters between visits in the control subjects. Furthermore, no differences in eosinophil parameters between allergic and control subjects were observed in the birch-pollen season (visit 1) and after the pollen seasons (visit 3) (Fig. 3). However, in the grass-pollen season (visit 2), all eosinophil parameters, except plasma ECP, were higher in the allergic subjects. No differences in total leukocytes between visits in both groups, and no differences between allergic and control subjects were observed (data not shown). In the birch- and grass-pollen seasons, the EOS correlated well with serum values of ECP and EPX ($r^2 = 0.40$ and 0.53 respectively, $P < 0.0001$). The correlation between serum ECP and EPX was $r^2 = 0.42$ ($P < 0.0001$). The relationship between EOS and plasma values was weak (plasma ECP: $r^2 = 0.09$, $P = 0.0036$; plasma EPX: $r^2 = 0.15$, $P = 0.0001$), as was the relationship between serum and plasma values (serum

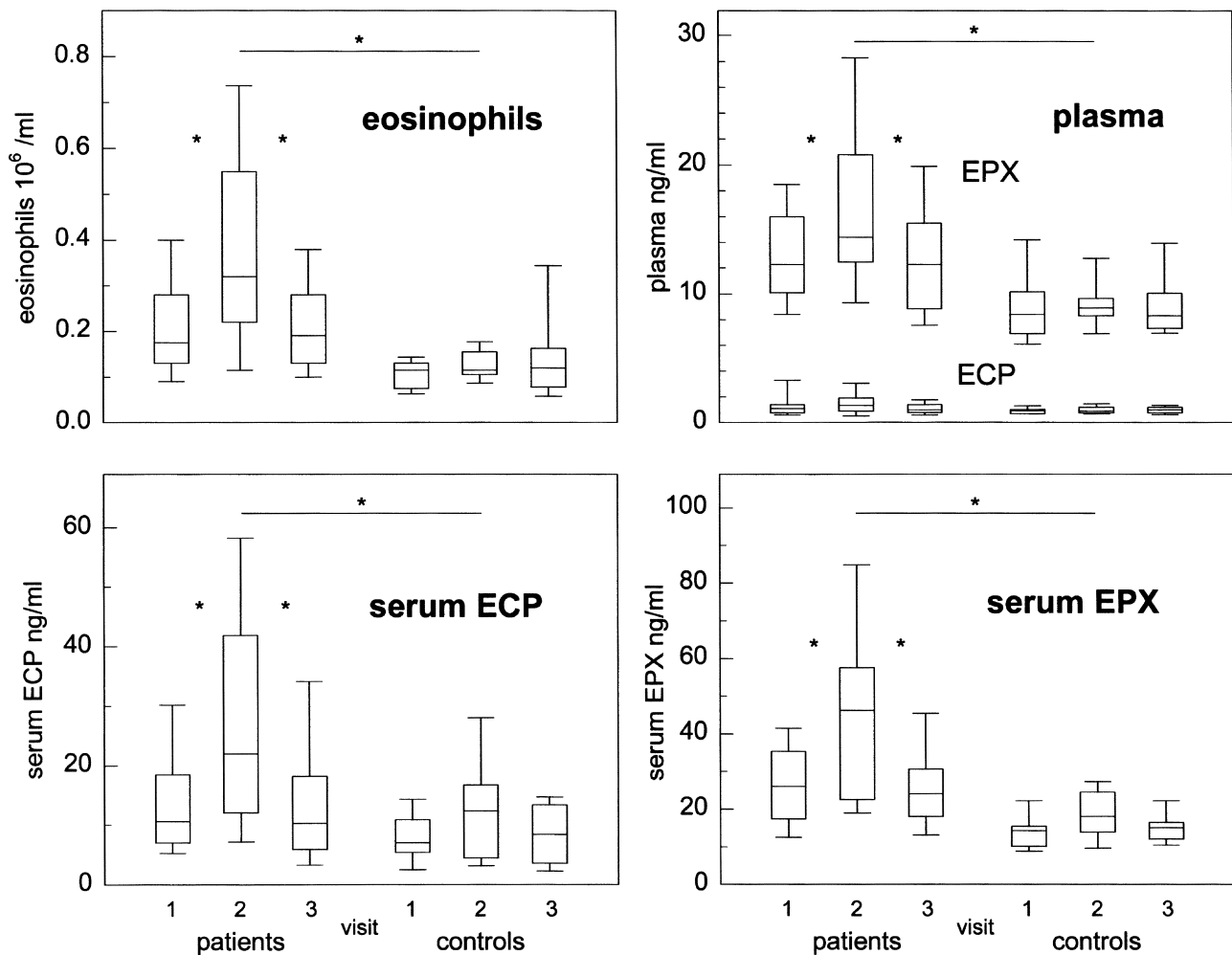


Figure 3. Seasonal changes in blood eosinophils, plasma EPX and ECP, serum ECP, and serum EPX of allergic patients and control subjects. Box-and-whisker plot showing 10, 25, 50, 75, and 90% cumulative relative frequencies (centiles). Visit 1) early birch-pollen season; visit 2) mid-grass-pollen season; visit 3) after pollen seasons. * $P < 0.05$

ECP vs plasma ECP: $r^2=0.05$, $P=0.037$; serum EPX vs plasma EPX: $r^2=0.15$, $P=0.0001$).

Relationship between symptoms, medication, and laboratory tests

As previously described, there was an intercorrelation between the different laboratory tests that would influence the interpretation of the usual Spearman regression correlations between symptom/medication score and laboratory tests. The PLS analysis solves this collinearity problem. It analyzes the variation in parameters between patients; i.e., if, for example, symptom scores change, do the HR tests change as well, and can the change in symptom scores be explained by the change in HR? Only results from visit 2 are presented. The results from visit 1 revealed the same pattern; however, the noise in the data was larger than at

visit 2. When we considered all symptom and medication scores (Y) and laboratory tests (X) together, a significant component was found (F-test value 40.2, correlation 0.34 for the cumulative relation, cross-validated); i.e., a significant relationship between symptom and medication score and laboratory tests was found. This component explained 46% and 19% of the variance in combined laboratory tests, and combined symptom and medication score, respectively; i.e., 19% of the total variation in symptom and medication scores was explained by 46% of the total variation in laboratory tests (Table 1). As the results indicate, factors other than our blood-test parameters influenced the severity of allergic rhinitis (and factors other than the severity of allergic rhinitis contributed to the changes in the blood-test results of our study).

The factor loadings and variance explained for each individual variable are shown in Table 1. It was seen that

Table 1. PLS analysis of *in vitro* tests (X), symptom scores, and medication scores (Y)

Variable	Factor loading	Variance explained
X (total)		0.46
Grass HR15	0.3339	0.78
Anti-IgE MAX	0.3200	0.72
Birch MAX	0.3164	0.70
Anti-IgE HR15	0.3159	0.70
Birch HR15	0.3154	0.70
Grass MAX	0.3013	0.64
Grass-specific IgE	0.2938	0.60
Birch-specific IgE	0.2404	0.40
Eosinophils	0.2297	0.36
Total IgE	0.2253	0.35
Serum EPX	0.2252	0.35
Plasma EPX	0.2066	0.29
Serum ECP	0.2034	0.28
Plasma ECP	0.1164	0.08
Y (total)		0.19
SYMP	0.7328	0.44
SEMP	0.5613	0.25
ANTI	0.3844	0.11
PRED	0.0113	0.00

the individual variance explained was higher than the cumulated, as the cumulated variance is a mean of both relevant (SYMP or grass HR15) and irrelevant (PRED or plasma ECP) parameters. The factor loadings show the relative significance of the variables in relation to each other, as defined by the PLS model (Table 1). For example, the relative weights of the dependent Y variables were 0.7328:0.5613:0.3844:0.0113, which can be normalized to 1:0.76:0.52:0.02. Estimation of a patient's total weighted symptom medication score then becomes:

$$1 \text{ SYMP} + 0.76 \text{ SEMP} + 0.52 \text{ ANTI} + 0.02 \text{ PRED}$$

The variance explained was largest for SYMP (44%), followed by SEMP (25%) and ANTI (11%), and no significant variance was explained for PRED. The accuracy of SYMP, SEMP, and ANTI predicted by the PLS analysis was good. The usual Spearman regression correlation coefficients of the predicted values plotted against the measured values were 0.90, 0.86, and 0.69 for SYMP, SEMP, and ANTI, respectively.

For the independent X variables, all histamine parameters were highly intercorrelated, and contributed about 70% of their total variance; i.e., 70% of the variation in HR could be

related to the variation in symptom-medication score, whereas 30% of the variation was explained by factors other than the rhinoconjunctivitis status of the patients. The results indicated that the symptom score and, to a minor degree, medication, were especially correlated with the HR results. The remaining laboratory tests were incorporated in the following order: specific IgE, eosinophil counts, total IgE, EPX, and ECP (Table 1). It was seen that eosinophil counts explained as much as the eosinophil-derived proteins in the blood and that plasma ECP was of no importance.

Discussion

Our study showed an increase, from the early birch-pollen season (visit 1) to the mid-grass-pollen season (visit 2), in specific IgE, basophil HR, and eosinophil parameters, indicating an activation/upregulation of the IgE synthesis, and the basophil and eosinophil leukocyte, from a low birch-pollen season with few allergic symptoms to a high grass-pollen season with increased allergic symptoms.

An increase in grass-specific IgE from visit 1 to visit 2 was observed, whereas total IgE did not increase. This indicates an antigen-specific activation of the IgE-producing B cell. Basophil leukocytes possess high-affinity receptors for IgE, and mediators are released when the allergen cross-links the specific IgE bound to its high-affinity receptor. We found an increase in both anti-IgE and specific IgE HR from visit 1 to visit 2. Furthermore, there was a clear HR relationship between the anti-IgE response and the allergen-specific response, which could be explained by a high proportion of specific IgE to the total IgE bound to the basophils. The grass parameters increased from visit 1 to visit 2, and, surprisingly, birch-specific IgE and birch HR also increased from visit 1 to visit 2. In our study year, the birch-pollen season started later than usual, and the blood tests at visit 1 were performed at the beginning of the birch-pollen season, when there was only a small amount of pollen. The birch-pollen load increased just after visit 1. A peak response in birch-specific IgE and birch HR *after* visit 1, which persisted for some time, could explain the observed increases in birch parameters from visit 1 to visit 2. Several studies have concluded that two independent variables control the IgE-mediated response in basophils: the amount of membrane-bound IgE needed to cause measurable HR (basophil sensitivity), and the subcellular factors regulating the degree of HR (basophil reactivity) (29, 30). The HR15 in our study is an expression of cell sensitivity, whereas MAX is an

expression of cell reactivity. The birch and grass MAX increased from visit 1 to visit 2, indicating an increase in basophil reactivity. As the MAX calculations are not expressed as "percent release of total cell histamine content", it cannot be excluded that the observed increase in basophil reactivity reflected an increase in the number of peripheral basophils. However, there was no difference in basophil counts between visit 1 and visit 2 (data not shown). In HR₁₅, only the birch response increased significantly from visit 1 to visit 2, whereas the increase in grass HR₁₅ did not reach statistical significance. The HR₁₅ to grass was high even at visit 1; i.e., the amount of membrane-bound grass-specific IgE was so high that the lowest concentration of the grass extract was sufficient to induce significant HR. If the cutoff (15 ng histamine/ml blood) was increased or lower grass-extract concentrations were used in the HR test, the patient results might have differed more, and the increase in basophil sensitivity have reached significance.

The macrophage-, T-, and B-cell interactions, and the mast-cell/basophil activation induced by an allergen give rise to release of cytokines. This has been associated with the activation and influx of eosinophils into the inflammatory microfocus (10, 31). This is in accordance with a study of *in vitro* eosinophil release which found a seasonal increase in peripheral eosinophil degranulation, indicating an activation of eosinophils (18). In our study, the allergic rhinitis patients showed an increase in blood eosinophil counts and serum ECP and EPX from visit 1 to visit 2, and a postseasonal decrease was found, supporting the concept of eosinophil upregulation and activation. However, the eosinophil counts were significantly correlated with serum ECP and serum EPX, and we did not find an individual increase in the ratios serum ECP/EOS and serum EPX/EOS from visit 1 to visit 2 (paired *t*-test, data not shown). Therefore, we cannot exclude the possibility that the increase in serum ECP and EPX observed in our study was partly due to an increased number of eosinophils. Several studies have demonstrated that the eosinophil plays a part in the allergic inflammation. A difference in eosinophils, serum ECP, and serum EPX between well-controlled nonallergic asthmatic patients and nonallergic control subjects, as well as between allergic asthmatic patients and nonallergic control subjects after the pollen season, has been reported (18, 19, 21). In another study investigating rhinitis patients vs controls, no difference in serum ECP was observed (32). Our study did not find any differences in eosinophils, serum ECP, and serum EPX after the seasons (or at the beginning of the birch-pollen season) between allergic rhinitis patients and healthy controls. The increased levels

in asthmatics, but not in rhinitis patients, could have been due to the difference in size of the diseased organ. Fifteen (31%) of the rhinitis patients in our study were also suffering from seasonal asthma. However, no differences were observed either after the season, or in the birch-pollen season between the asthmatic rhinitis patients, the rhinitis patients without asthma, and the control subjects, and no difference was found between the asthmatic rhinitis patients and the rhinitis patients without asthma during the grass-pollen season (data not shown).

We found no differences in eosinophil parameters and basophil HR (except birch HR) of the allergic patients between visit 1 (birch season) and visit 3 (after the seasons). The birch-pollen load was low before visit 1, and the patients had few symptoms. On the other hand, the eosinophil and basophil HR parameters were increased during the peak grass-pollen season, with a high pollen load and with symptoms in all patients. These results could indicate that the severity of the allergic rhinitis is influenced by the eosinophil and basophil leukocyte.

The production of specific IgE and subsequent sensitization of mast cells and basophils are important factors involved in the pathophysiology of allergic rhinitis, and eosinophils participate in the local allergic inflammation, but it is presently not known to what extent the different parameters can explain disease severity. The partial least square PLS analysis has previously been used to describe the relationship between air-pollution parameters and plant-growth parameters (28). In our study, the PLS analysis may possibly explain to what extent the different *in vitro* parameters express the severity of allergic rhinitis.

The accuracy of the symptom and medication scores predicted by the PLS analysis was high, showing a means to combine a symptom and medication score in an investigator-independent way. We found a correlation among most of the different *in vitro* parameters; therefore, the PLS analysis seems a good choice, as this multivariable model solves the collinearity problem (intercorrelation between variables). The model showed a significant relationship between clinical symptoms and the blood-measurement markers of immunoinflammation, with an indication of different importance among the individual tests. When looking at symptom score and medication, the variance explained was higher for the symptoms than the medication. This could be explained by the patients' attitude toward symptoms and medication. In another study, we followed patients during both pollen seasons and found that they tended to use as little medication as possible in order to control symptoms to a certain individual level (data to be

published). The prednisolone seemed to be of no importance, perhaps because only 10 patients in our study used prednisolone, and the mean daily prednisolone consumption was low. We did not find differences in any blood-test parameters between the allergic patients with or without prednisolone treatment at the three visits, again, presumably, because of the minimal prednisolone medication. However, several studies have shown that glucocorticoids affect the allergic inflammation directly by cell receptors, or indirectly by disruption of the cytokine network; e.g., the eosinophil generation, survival, and function [33]. The PLS-analysis results of the relationship between symptom and medication scores and the different laboratory tests showed that a few selected tests tell as much as all the tests put together. The HR test seemed to be the most relevant parameter of clinical symptoms during the pollen season. However, although a significant relationship was found between *in vitro* tests and the symptom and medication scores, the PLS model explained only less than half of the total variation in symptoms and less than one-quarter of the total variation in medication. This indicates that blood parameters do not necessarily reflect the actual disease status of the target organ. The measurements of number of

eosinophils, concentration of ECP/EPX in the tissue, and the releasability of tissue basophil/mast cells might have increased the percentage of the total variation in disease severity explained by these parameters. Other tissue immunologic cells such as CD4⁺ T-helper cells [7], Langerhans' cells [34], and endothelial cells [35, 36] have been shown to be related to allergic inflammation, indicating that allergic inflammation and its relation to the severity of the allergic disease is very complex.

In conclusion, a seasonal increase in the blood measurements of basophil HR, eosinophils, serum ECP and EPX, and specific IgE was observed in allergic rhinitis patients. A relationship was observed between the severity of allergic rhinitis and these laboratory tests, and the basophil HR test proved to be the most relevant test. However, the present study indicates that other factors contribute to the severity of allergic rhinitis.

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