

Seasonal differences of peak expiratory flow rate variability and mediators of allergic inflammation in non-atopic adolescents

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Variations in peak expiratory flow (PEF) and serum eosinophil mediators were studied in healthy adolescents. Twenty-five boys and 31 girls, 11–16 years of age (mean age 14.3 years), were selected and investigated during the birch pollen season of 1995; 45 were also investigated during the autumn of the same year. The PEF was measured twice daily and eosinophil mediators in serum and in urine were measured by radioimmunoassay (RIA) once during the birch pollen season and once in autumn. The type values of the daily PEF variation, expressed in amplitude percentage mean, were 6.4 and 3.9%, mean values were 7.35 and 6.74%, and the 95th percentiles were 18 and 14%, during the birch pollen season and autumn, respectively. The 95th percentiles were 41 and 38 µg/l for serum eosinophil cationic protein (s-ECP), 74 and 62 µg/l for serum eosinophil protein X (s-EPX), 987 and 569 µg/l for serum myeloperoxidase (s-MPO), and 165 and 104 µg/mmol for urinary eosinophil protein X/urinary creatinine (u-EPX/u-creatinine), during the birch pollen season and autumn, respectively. The levels of the eosinophil mediators decreased significantly from May (n=56) to November (n=45), for s-ECP from a median value of 14 µg/l to 7 µg/l ($p=0.001$), for s-EPX from a median value of 28 µg/l to 20 µg/l ($p=0.001$), and for the neutrophil mediator, s-MPO, from a median value of 440 g/l to 292 g/l ($p<0.001$). The PEF variability decreased significantly ($p=0.037$), from spring (n=55; median 8%, 95% confidence interval [CI] 7.8–10.19) to autumn (n=44; median 6%, 95% CI 6.1–8.9). A significant correlation was found between the levels of s-ECP and s-EPX ($r_s=0.7$, $p<0.001$), between s-ECP and s-MPO ($r_s=0.6$, $p<0.001$), between s-EPX and s-MPO ($r_s=0.4$, $p<0.005$), and between s-EPX and u-EPX/u-creatinine ($r_s=0.6$, $p<0.0001$), in the birch pollen season (n=56) and in the autumn (n=45). There was a positive correlation found in PEF variability between the two seasons (n=43; $r_s=0.5$, $p=0.0006$). No other correlation was found between PEF variability and any other parameters. The difference in the levels of eosinophil mediators between seasons in non-atopic, healthy children is unexplained. Normal limits for mediators were higher and PEF variability was almost the same as has been reported in adults. When using normal values, seasonal influences should be considered.

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The diagnosis of asthma is based mainly on medical history, physical examination, and lung function. Serial measurements of peak expiratory flow rate (PEF) are often used in the clinical man-

agement of asthma to aid diagnosis, to assess severity, and to determine the influence of environmental factors (1). A variety of methods for the assessment of PEF variability have been used. The diurnal variability is often calculated as either daily amplitude percentage of highest (2–5) or daily amplitude percentage of mean (6–8).

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Eosinophil mediators found in the serum, such as serum eosinophil cationic protein (s-ECP) and serum eosinophil-derived neurotoxin (s-EDN), or a mediator found in the urine, urinary eosinophil protein X (u-EPX), may more directly reflect the inflammatory process in asthma (9,10). The s-ECP, s-EPX, and u-EPX levels are increased in asthmatic children compared with healthy controls (11–16). Also, the level of s-ECP has been used for monitoring the inflammatory process (11,15) and to assess asthma severity in asthmatic children (17). It has even been suggested that elevated s-ECP may identify future asthmatic children (18).

Urinary-EPX could be another useful marker in monitoring childhood asthma (14,16,19). A significant decrease in u-EPX was found in atopic asthmatic children after 3 months of treatment with anti-inflammatory medication (16,19).

Myeloperoxidase (MPO) is a neutrophil-derived protein that is assumed to reflect neutrophil activity, but it is also found in monocytes (20). The role of this protein in allergic inflammation is unclear. In the serum of asthmatic children, elevated serum MPO (s-MPO), together with elevated s-ECP and s-EPX, has been interpreted as a sign of on-going inflammation (21,22). Raised levels of s-MPO have been found in asthmatic children with bacterial infections (23). In our previous study, values of s-ECP, s-EPX and s-MPO, and PEF variation, differed between the birch pollen season and out of season, in birch- and grass pollen-allergic children with rhinitis (24). Carlson et al. found higher s-ECP, s-EPX, and s-MPO values during the pollen season in birch pollen-allergic patients with asthma than before the pollen season (25).

To correctly interpret data obtained during 5 years of monitoring (during and between seasons) of children included in the PAT (Preventive Allergy Treatment) study (26), normal values were needed. The aim of our study was therefore to investigate the normal variation of these variables in healthy, non-atopic Swedish adolescents, during the birch pollen season and during the autumn, and to investigate whether these normal values were the same in both seasons.

Materials and methods

Subjects/selection criteria

In May 1995, 61 adolescents (26 boys and 35 girls) were invited to participate in the study. They were between 11 and 16 years of age (mean age 14.3 years). One was 11 years of age, two were 13 years of age, 38 were 14 years of age, 19 were 15 years of age and one was 16 years of age. The study group

comprised those children who answered 'no' to the following questions:

1 Have you: (i) had asthma; (ii) had wheezing; (iii) had chronic cough; (iv) had a blocked nose; (v) taken any medication for any of these diseases; (vi) been a smoker?

2 Have you ever heard from your doctor that you have/had: (i) asthma; (ii) asthmatic bronchitis; (iii) hay fever; (iv) atopic dermatitis; (v) food allergy/intolerance; (vi) allergic urticaria/itchy rash; (vii) parasitic infection; or (viii) urinary tract infection?

All adolescents were studied during the birch pollen season and 51 were also studied during the autumn season. Thus, based on their own evaluation, they were selected as asymptomatic, non-allergic adolescents. To confirm their non-atopic status, birch-specific immunoglobulin E (IgE) and Phadiatop[®] tests were carried out. Low-grade birch-specific IgE levels of >0.35 kU/l were present in five samples (0.57, 0.77, 0.91, 1.75, and 4.18 kU/l), and Phadiatop was positive in 11 samples. Adolescents with positive birch-specific IgE were excluded during the birch pollen season, and those with a positive birch radioallergosorbent test (RAST) or Phadiatop (n=16) were excluded from the calculations of the PEF variability during the autumn season. Thus, 56 adolescents (25 boys and 31 girls) were included during the birch pollen season and 45 (16 boys and 25 girls) during the autumn. Their mean age was 14.3 yr.

Collection of serum and urine

During the last week of May and the last week of November, a blood sample was taken from each participant between 08.00 h and 16.00 h. Blood was collected in Vacutainer SST tubes and stored at room temperature (20–22°C) for 60±10 min, centrifuged at 1500 g for 10 min and then stored at –20°C until assayed.

Urine was voided just before blood sampling. It was transferred to 2-ml vials and stored at –20°C until assayed. Analyses were carried out by Pharmacia & Upjohn Diagnostics AB (Uppsala, Sweden). All samples were analyzed at the same time.

Birch-specific IgE

Birch-specific IgE was determined by the Pharmacia CAP[®] system, according to the manufacturer's instructions (Pharmacia & Upjohn Diagnostic AB). Birch allergen-specific IgE values of 0.35 kU/l, corresponding to CAP RAST class 1 or greater, were considered positive. All sera were assayed in duplicate and the mean values were estimated.

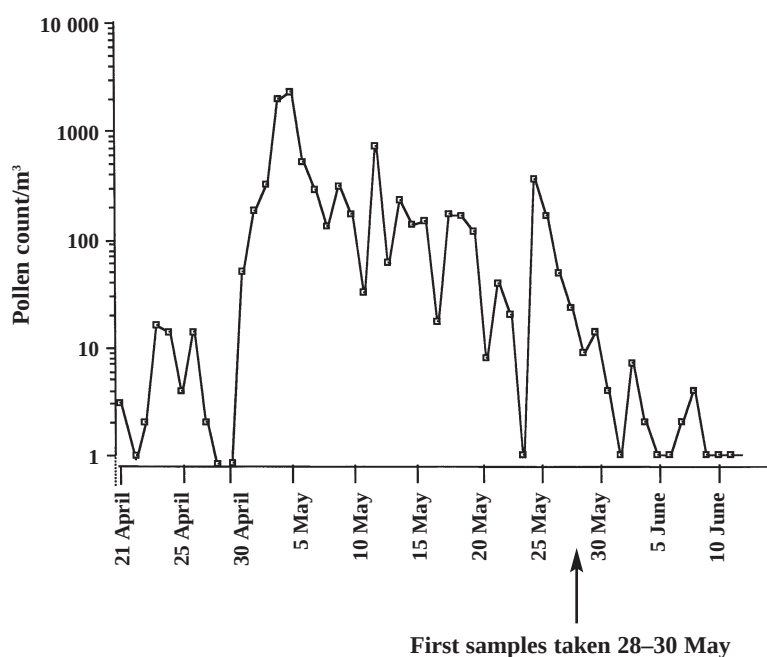


Fig. 1. Pollen counts/m³ during the period April 21 to June 10, 1995. The arrow pointing on the x-axis indicates the date that the samples (blood and urine) were collected and peak expiratory flow rate (PEF) recorded papers from the participants in May.

Phadiatop

The Phadiatop test is a screening assay using a disc coated with a combination of common inhalant allergens. The test was performed according to the manufacturer's instructions (Pharmacia & Upjohn Diagnostic AB). The test is qualitative and the results are given as 'positive' or 'negative'.

Determination of mediators of inflammation

Values of s-ECP, s-EPX, and s-MPO were measured by using radioimmunoassays (RIAs) from Pharmacia Diagnostics AB (27–29). The intra- and inter-assay coefficients of variation (c.v.) were both <3% for all three mediators investigated. The upper limit for the normal levels in adults, i.e. 95% percentile in non-atopic individuals, according to the manufacturer is 11.8 µg/l for s-ECP (28), 38.5 µg/l for s-EPX (29), and 634 µg/l for s-MPO (29).

Eosinophil protein X (EPX) was measured in urine using the Pharmacia EPX RIA (Pharmacia & Upjohn Diagnostics AB), according to the manufacturer's instructions. Urine samples were diluted 1 in 10 in a phosphate buffer, pH 7.4, containing 0.15 M NaCl, 1% bovine serum albumin (BSA), 0.1% Tween-20, 10 mM EDTA (di-sodium salt) and 0.2% CTAB (*N*-cetyl-*NNN*-trimethyl-ammoniumbromide). Inter- and intra-assay variation was less than 10% and the detection limit was 3 µg/l. Creatinine in urine (u-creatinine) was assessed using the alkaline picrate method (Jaffés reaction, HiC Creatinine; Boehringer Mannheim GmbH, Mannheim, Germany). The u-EPX/u-creatinine ratio (µg/mmol) was calculated. The upper limit for the normal variation of the ratio u-

EPX µg/ u-creatinine mmol in adults is 118 µg/mmol (28).

Recording of PEF variability

For 4 weeks during the birch pollen season in May and 4 weeks in autumn (from October 30 to November 26), all the children recorded their peak expiratory flow using a Mini Wright peak-flow meter. The same PEF meter was used throughout the study. The best value of three attempts was recorded.

PEF variation was expressed in two ways – amplitude percentage highest and amplitude percentage mean (1) – according to the following formulas:

Amplitude percentage highest

$$= \frac{[\text{Highest PEF} - \text{lowest PEF} (\%)]}{\text{Highest PEF of the day} (\%)}$$

Amplitude percentage mean

$$= \frac{[\text{Highest PEF} - \text{lowest PEF} (\%)]}{\text{Mean of the day PEF} (\%)}$$

The type value of PEF variation, i.e. the value that appeared most often as a 'common value' for every individual, was also of clinical interest.

Scoring of symptoms during the season

Symptoms were recorded weekly, during the same period as PEF values were recorded. The children were questioned regarding symptoms of asthma and hay-fever. They were asked to answer the questions, 'Have you had any symptoms of asthma

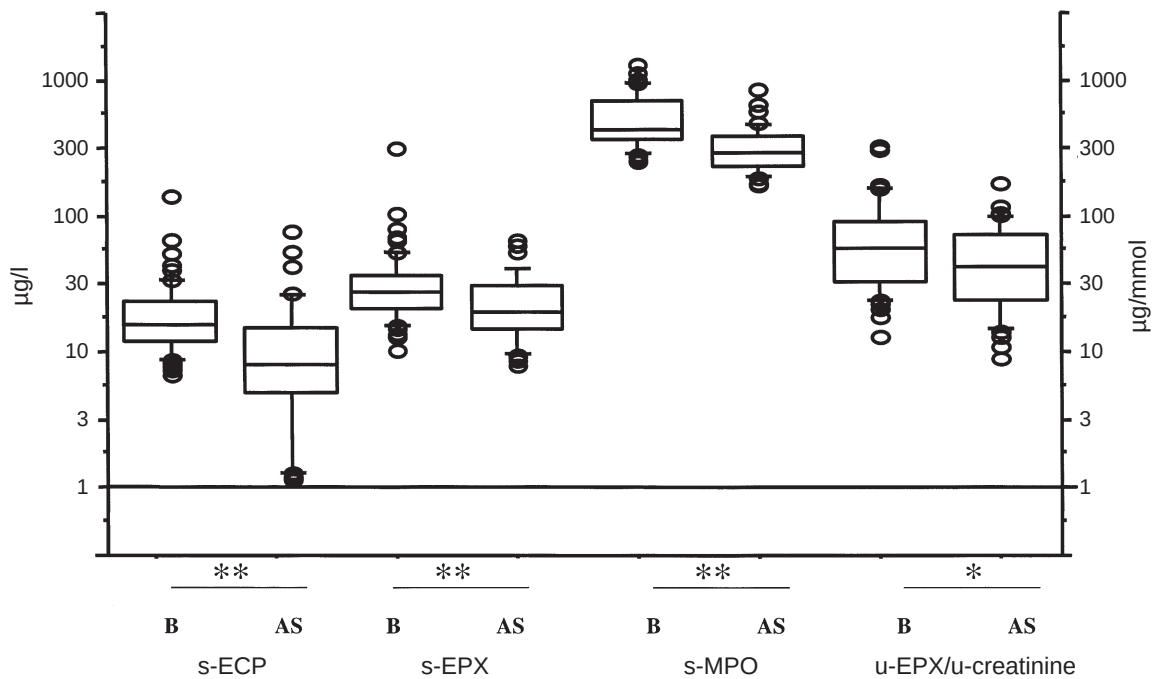


Fig. 2. Serum levels of eosinophil cationic protein (s-ECP), eosinophil protein-X (s-EPX), and myeloperoxidase (s-MPO), were significantly higher ($p = 0.001$), and the eosinophil protein X/urinary creatinine (u-EPX/u-creatinine) ratio was significantly higher ($p = 0.003$), during the birch pollen season than in the autumn.

this week' and 'Have you had any symptoms of hay-fever this week'. They were also asked to grade these symptoms by marking on a visual analog scale (VAS) of 100 mm, an appropriate point between 'no symptoms' (0) and 'very much symptoms' (100). The distance in mm from no symptoms was used in the calculations as a measure of symptom severity. They were also asked to note any fever, infection or illness experienced during the study.

Pollen counts

Birch pollens were counted from April to July 1995. A Burckard pollen trap was on the roof of the Swedish Meteorological and Hydrological Institute in Norrköping, located ≈ 45 km from the school of the children who participated. Birch pollen counts of $>100/\text{m}^3$ were considered as the limit for symptoms. Such high levels were recorded from the May 3 to 27 May 1995. The peak value of 2,285 birch pollen/ m^3 was found on May 6. The pollen curve for 1995 is shown in Fig. 1.

Ethics

The Ethics Committee of the University of Linköping approved the study (registration number 95115).

Statistical analysis

Serum and urinary levels of mediators were log transformed. Changes in parameters within the

birch pollen season and autumn were analyzed by using the Wilcoxon paired test, and correlations between groups were calculated by using Spearman Rank Correlation analysis. For the PEF, amplitude percentage mean value and amplitude percentage highest were calculated and the upper limit of the normal variation was expressed as the 95th percentile of the 95th percentile of the individual variations. The type value was calculated from the amplitude percentage highest and expressed in the same way. As one child had lost his PEF report during the birch season, he was excluded from both seasons in the PEF statistical comparison. The upper limit of the normal variation was expressed as the 95th percentile for the s-ECP, s-EPX, s-MPO, and u-EPX/u-creatinine during the birch and autumn seasons, respectively.

Results

Symptoms and sensitization

No participant reported symptoms of asthma or hay-fever, or had evident symptoms of on-going infection, during the birch pollen season or in the autumn.

A birch-specific IgE level of >0.35 kU/l was present in five (one male, four females) serum samples, and Phadiatop was positive in 11 (six males, five females) serum samples, as shown in Table 1.

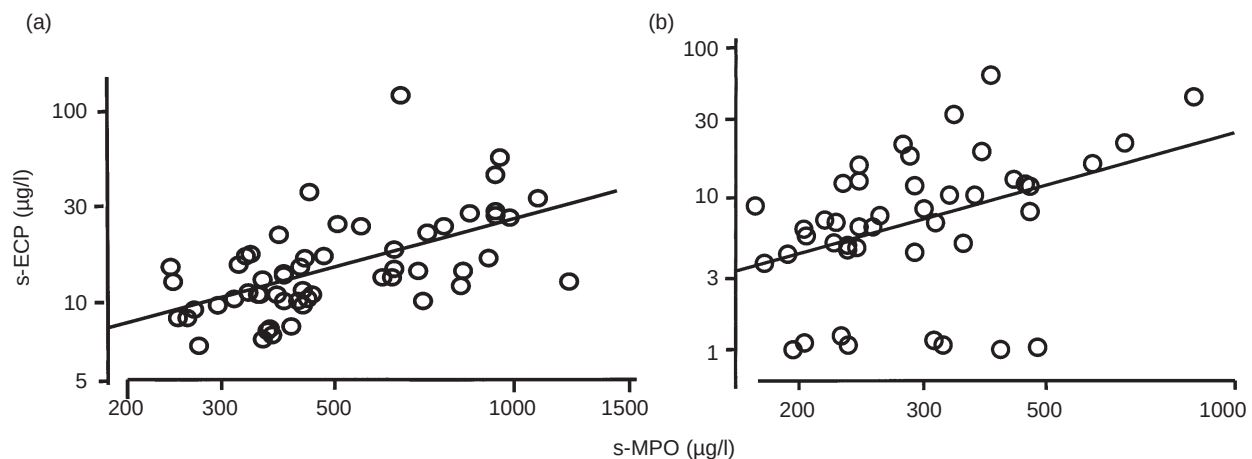


Fig. 3. (a) Correlation between the levels of serum eosinophil cationic protein (s-ECP) and serum myeloperoxidase (s-MPO) in the birch pollen season. (b) Correlation between the levels of s-ECP and s-MPO in the autumn.

Mediators of allergic inflammation

The median level, 95% confidence interval (CI), range and 95th percentile for s-ECP, s-EPX, s-MPO and u-EPX/u-creatinine are given in Table 2.

Seasonal variation

The data from the 45 adolescents tested in both May and the autumn showed a significant decrease in the autumn for s-ECP, s-EPX, s-MPO (all $p < 0.0001$), and u-EPX/u-creatinine ($p = 0.0033$).

The seasonal variation is shown in Fig. 2.

Comparison with the levels in adults

The values reported in the adolescents tended to be higher than normal values of adults, as reported by Olofsson et al. (28), Pharmacia & Upjohn Diagnostics AB (data on file). The s-ECP level was higher than the upper limit of the normal level in adults (11.8 µg/l) in 36/56 and 15/45 adolescents during the birch pollen season and during the autumn, respectively.

The s-EPX level was higher than the upper limit of the normal level in adults (38.5 µg/l) in 14/56 and seven of 45 adolescents during the birch pollen season and during the autumn, respectively.

The s-MPO value was higher than the upper limit of the normal level in adults (634 µg/l) in 19/56 adolescents during the birch pollen season but only in two of 45 adolescents during the autumn.

The u-EPX/u-creatinine ratio was higher than the upper limit in adults (118 µg/mmol) in 10/56 adolescents in the birch pollen season but in none during the autumn season.

Correlation

A significant correlation was found between the levels of s-ECP and s-EPX ($r_s = 0.7$, $p < 0.001$), s-ECP and s-MPO ($r_s = 0.6$, $p < 0.001$), s-EPX and s-MPO ($r_s = 0.4$, $p < 0.005$), and s-EPX and u-EPX/u-creatinine ($r_s = 0.6$, $p < 0.0001$), in the birch pollen season ($n = 56$) and in the autumn ($n = 45$).

The correlation between s-ECP and s-MPO in the birch pollen season and in the autumn is shown in Fig. 3(a), 3(b).

Table 1. Characteristics of the schoolchildren/adolescents tested and excluded subjects (mean age 14.3 years, range 11–16 years) during the birch pollen season (BPS) and in the autumn

				Gender	
		Number	%	Male	Female
Total children	BPS	61	100	26	35
	Autumn				
Specific IgE to birch (5)	Remaining (not birch sensitive) in BPS	56	92	25	31
Phadiotrop positive (7)	Remaining in autumn	45	87	18	27
Positive tests	Phadiotrop positive	11	18	6	5
	Specific IgE to birch	5	8	1	4
		4	36		2
Did not participate in the autumn, with positive test	Phadiotrop positive specific IgE to birch	2	40	2	2
Did not participate in the autumn, with negative test		5	8	4	1

IgE, immunoglobulin E.

Table 2. Variability of peak expiratory flow rate (PEF) and mediators of allergic inflammation in healthy and sensitized schoolchildren/adolescents

	Healthy					Sensitized			
	n	Median	95th p.c.	95th CI*	Range	n	Median	95th p.c.	Range
PEF variability % of highest within the day									
Birch season	55	8	17	8–10	2–22	5	9	13	8–13
Autumn season	44	6	13	6–9	2–28	7	6	16	4–19
s-ECP (µg/l)									
Birch season	56	14	41	15–24	6–123	5	14	18	6–18
Autumn season	45	7	38	9–16	4–68	7	8	28	6–36
s-EPX (µg/l)									
Birch season	56	28	74	25–48	10–317	5	29	34	22–35
Autumn season	45	20	62	20–35	8–163	7	26	52	14–61
s-MPO (µg/l)									
Birch season	56	440	987	480–616	256–1298	5	524	538	297–541
Autumn season	45	292	569	281–364	170–855	7	256	564	163–650
u-EPX/u-creatinine (ug/mmol)									
Birch season	56	58	165	59–93	13–332	5	46	68	40–72
Autumn season	45	43	104	40–61	9–178	7	53	105	18–105

s-ECP, serum eosinophil cationic protein; s-EPX, serum eosinophil protein-X; s-MPO, serum myeloperoxidase; 95th CI, 95th confidence interval; 95th p.c., 95th percentile.

* Not given for sensitized group owing to small group size.

PEF variability

Almost all adolescents recorded their PEF for all days during the required 28-day time periods (average 26.5) during the birch pollen season and in the autumn. One child recorded for only 17 days and one child lost his recording paper during the birch pollen season. During the autumn, one child reported the same PEF result every time. With the exception of this child's PEF sheet, all PEF sheets appeared to be in order.

The PEF variation seemed to be higher during the birch pollen season than in the autumn. Sixty-four per cent of the adolescents had a higher PEF variation in May and 36% had higher PEF variation in the autumn. The type value for PEF variation (expressed as amplitude percentage highest) during a day in May (n=55) was 6%. The median value during the birch pollen season was 8% and the 95th percentile was 16.69%. During autumn (n=44), the type value was 3.8%, the median was 6.49%, and the 95th percentile was 13.28%.

The type value for PEF variation (expressed as amplitude percentage mean), in May (n=55) was 6.4%, the mean was 7.35%, and the 95th percentile was 18.16%. During autumn (n=44), the type value was 3.9%, the mean was 6.74%, and the 95th percentile was 14.24%.

Adding the values from sensitized adolescents to the group did not change the PEF variation data significantly.

The median values and upper limits for the normal variation (the 95th percentile), and 95% CI for PEF variability during the birch and autumn seasons, are given in Table 2.

Seasonal variation

The PEF variability also decreased significantly ($p=0.037$), from the birch pollen season (n=55; median 8%, 95% CI 7.8–10.19) to autumn (n=44; median 6%, 95% CI 6.1–8.9).

Correlation

There was a positive correlation in PEF variability between the two seasons (n=43; $r_s=0.5$, $p=0.0006$). No other correlation was found between PEF variability and any other parameters.

Discussion

Initially we defined healthy children as those who had no history of known allergy or asthma or signs of on-going infection. Despite having no symptoms of atopy, five children had positive specific IgE against birch and 11 children were positive on Phadiatop testing. However, the levels were low, which may explain the absence of clinical symptoms. Therefore, to avoid any possible influence by atopy on normal values, we defined 'non-atopic', based on the patient's subjective evaluation, as absence of specific IgE against birch and a negative Phadiatop test.

We found the levels of all the mediators to be significantly higher during the birch pollen season than in the autumn. This was true even when the Phadiatop- and birch IgE-positive adolescents were excluded from analyses of results from both the birch pollen season and the autumn. One possible explanation for the difference in mediator

levels between seasons might be that temperature influences the release of mediators from eosinophils. Wantke et al. (29) found that the s-ECP level did not significantly decrease despite storage of serum at room temperature for 24 h. The room temperature in our laboratory varied minimally, from 20 to 22°C during the year, and therefore temperature variation cannot explain the differences found in our present study between the two seasons. All blood samples were analyzed at the same time to avoid laboratory errors.

There was a clear seasonal variation in the levels of inflammatory markers in our study group of healthy adolescents, which has not been reported previously. The explanation for higher values in our study is not clear; however, there may be two reasons. First, pollen causes the release of a variety of enzymes, including proteases, that are able to cause detachment of airway epithelial cells from their substratum *in vivo* (30). These proteases might promote a mild inflammation in some of all subjects during the birch pollen season (31). Second, the increased levels of inflammatory markers might be the result of inflammatory responses mediated by antibodies to birch, such as IgG₁ and IgG₄. The presence of such antibodies has been found in young children in Sweden (32). It was found that the levels of IgG sub-class antibodies peaked in children at 18 months then decreased to normal levels by 8 years of age in both atopic and non-atopic children. Those children were not followed-up after reaching 8 years of age.

Which season's value should be taken as the 'normal'? As some unknown factor(s) may cause higher levels of mediators and PEF variability during the birch pollen season, we believe that the levels of mediators and PEF variability in the autumn should be considered as 'normal'.

The normal levels of mediators of inflammation were higher than the levels reported in adults (27, 28). In infants, the levels are higher than in adults, but slowly decrease after the second year of life (33). Carlsen reported in his recent review that significantly higher levels of s-MPO were found in healthy infants younger than 12 months of age than in children of 12–23 months of age and in children of 24–41 months (34). However, prospective studies among school-age children are lacking.

The year of 1995 was a 'moderate-to-severe' birch pollen year compared to 1992, 1993, and 1994. During 1992, the highest birch pollen count was 367 pollens/m³. In 1993, the highest birch pollen count was 5,793 pollens/m³. In 1994, pollen counts did not exceed 100 pollens/m³. In 1995, the highest birch pollen count was 2,285 pollens/m³,

recorded on May 6. However, no-one has yet reported whether the level of inflammatory mediators and PEF variability in healthy individuals is dependent on pollen counts.

We found a significant correlation within eosinophil mediators. The reason for this was obvious because all originate from eosinophils. However, we also found a significant correlation between eosinophil mediators, i.e. s-ECP or s-EPX, and neutrophil mediators, i.e. s-MPO. The reason for this might be genetic. Both the EPX gene and MPO gene consist of 12 exons and 11 introns, located on the long arm of chromosome 17 at band 17q23.1 (35–37). Therefore, the organization of the EPX gene is similar to that of the MPO gene. At the amino acid sequence level, EPX shows homology with MPO (69%). Therefore, EPX is generated, together with MPO, from an ancestral gene on the same locus. The EPX gene is located 34 kb from the MPO genes and they form a cluster within a 90 kb region, aligned in a tail-to-tail manner. They are both generated by duplication of a common ancestral gene on chromosome 17 (38). However, analytical tests for ECP, EPX, and MPO show no cross-reaction (Pharmacia AB, internal report).

Compliance is difficult to achieve, both with the use of diaries and measurements of PEF. Therefore, this aspect of our study was considered in detail. Various asthma studies had been reported by the press and the media before and during this study. As a result, almost all of the schoolchildren who participated were familiar with the PEF meters. A child of the headmistress from the school took part in our previous study. Support from the school was good. Proper instructions for using the PEF meter were painstakingly and repeatedly given to the children. The PEF recorded sheets and the symptoms sheets were collected individually, and each child was asked whether they had any problem completing it. Except for one child who reported the same PEF result every time, all PEF recording sheets appeared to be accurate. Thus, the response rate was good in our study.

Quackenboss et al. (39) reported on 171 children, 6–14 years of age. In their population study, the median of the amplification percentage mean PEF for non-asthmatic children was 8.2%, with 31% representing the 95th percentile for the population. All the ages were, however, grouped together. The subjects were asked to make their PEF measurements from two to four times a day. Our group of children recorded their PEF twice a day. Moreover, the questionnaire of their study was different from the one of the present study.

Hetzel & Clark, in 1980, suggested (40) that a PEF of $\geq 20\%$ of the mean value was a useful screening test-point for asthma in adults. They found that the mean PEF amplitude in normal adults was $8.3 \pm 5.2\%$. In addition, they reported that 66% of healthy adults showed a significant circadian rhythm in PEF. In the present study, we found a similar, normal PEF variability, which varied in non-atopic adolescents from 18% during the birch pollen season, to 14% during the autumn season. To our knowledge, no seasonal variation in PEF variation has been reported in healthy adults.

We can therefore conclude from this study that non-atopic, healthy adolescents, of between 14 and 15 years of age, have higher levels of s-ECP, s-EPX, s-MPO, and u-EPX/u-creatinine than that previously reported in adults. The PEF variability in healthy adolescents is similar to that of adults. There is a significant difference in the levels of the mediators between May (birch pollen season) and November. As a reference, the values found during the autumn are probably most appropriate.

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