

Seasonality of sputum eosinophilia in adolescents with asthma remission: effects of montelukast

To the Editor,

Remission of asthma may occur at any age, but most commonly presents during adolescence and young adulthood. Eosinophilic inflammation is the predominant type of inflammation in asthmatic children and adolescents—also present during the remission of asthma.^{1,2} The persistence of eosinophilic inflammation is a risk factor for recurrence of asthma in adulthood and the reduction in lung function.¹ Although montelukast is a much less effective anti-inflammatory agent, there are some observations that it could impact airway remodelling present in asthma remission and perhaps can reduce the incidence of asthma recurrence.³ There are no long-term studies of eosinophilic inflammation in asthma remission taking into account seasonality or the impact of montelukast on parameters of inflammation.

The aim of our study was to evaluate eosinophilic inflammation in adolescents with clinical remission of asthma and to assess the effect of the montelukast.

A yearlong, prospective, double-blinded, placebo-controlled cross-over study was performed at the Allergy Outpatients Clinic of the Regional Hospital in Lesko, Poland.

Participating subjects 14–18 years of age were in short-standing remission, under the care of the outpatient clinic. Patients were classified to be in clinical remission of asthma if they were free of symptoms for at least 1 year without receiving ICS and other treatment intervention.⁴

Study design. The protocol of the study was approved by the Bioethics Committee of the National Institute of Tuberculosis and Lung Disorders (approval number KB 68/2010). Parents/guardians of all patients and all patients over 15 years of age gave written informed consent before the start of participation. The primary end-point in our study was to evaluate eosinophilia in induced sputum of patients in asthma remission (in both montelukast and placebo groups). The secondary end-points of the study were clinical parameters, spirometry and airway hyper-reactivity. Measurements included the following:

Daily monitoring of clinical asthma symptoms to obtain the number of days with the presence of symptoms and days taking medication.

Monthly spirometry (EasyOne—Medizintechnik Schweiz).

Quarterly assessment of induced sputum and airway hyper-responsiveness upon challenge with hyperosmolar salt. Induction of sputum and measurement of AHR were performed at a single stage using the combined method as previously described.⁵ Abnormal sputum was defined as an eosinophil ratio above 2.5%.

All patients received ML for a 6-month period, in a cross-over manner, as the experimental study consisted of two cohorts: those who received montelukast for the first 6-month period (cohort ML-PI) and those who received a placebo (PI) for the first 6 months (cohort PI-ML). The first study period lasted from September to March, the second from April to August.

The following stratification criteria were used to randomly assign patients to the study groups: age and values of FEV₁, DRS and sEos. Medications were prepared in the hospital pharmacy as individual white capsules. Participants' adherence to the treatment regimen was evaluated periodically by the number of issued capsules each month.

From a pool of 83 asymptomatic asthmatics under care selected based on a retrospective analysis of outpatient files, 44 participants were initially enrolled (cohort ML-PI n = 24, cohort PI-ML n = 20).

Finally, 41 subjects (17.0 ± 1.2; mean age ± SD) underwent a full annual cycle of clinical observation. The participants were in a short-standing remission of asthma (12–36 months; an average of 19 months), with a clear predominance of male subjects (71%). All were atopics: forty of the participants (97.5%) were allergic to dust mites, fifteen (36.5%) to grass pollen, four (9.7%) to tree pollen and three (7.3%) to furred mammals' allergens.

The initial assessment was carried out at the end of summer (August). There were no differences in the duration of remission, initial values of sEos (sputum eosinophilia), DRS (dose-response slope) or spirometry between the two cohorts at the start of the study.

The following assessments were performed at quarterly intervals: in the autumn (after 3 months), in the winter (after 6 months), in the spring (after 9 months) and in the summer (after 12 months).

For those who completed study, the overall treatment adherence for montelukast or placebo was 93% by capsules count.

There was a significant association between the content of sputum eosinophils in both study groups and seasonality. In the first half of the yearlong observation period, all study subjects exhibited a trend of increasing sEos values. In the second half of the observation, a sustained decrease in sEos values was observed in all study subjects (Table 1). There was an elevated sputum eosinophils count in at least one assessment in 66% of the patients (from 20% in the summer to 53% of subjects in the autumn). Elevated values of sEos were observed in all measurements in 4 patients. Elevated sEos values were present 4 times in 3 patients, 3 times in 7 patients, 2 times in 4 patients and in only one measurement in 9 patients; always normal sEos were observed in 14 patients.

TABLE 1 Description of patients included in the study

	First placebo (n = 18)	First ML (n = 23)	P
Age (y)	16.6 (±1.1)	17.3(±1.1)	.04
Sex (F/M)	6/12	6/17	.61
Age of asthma onset (y)	5.2 (±2.3)	5.3 (±2.5)	.93
Asthma duration (y)	9.4(±2.3)	10.0 (±3.4)	.39
Duration of asthma remission (mo)	19.8 (±7.8)	18.5 (5.7)	.75
sEos (%)	1.0 (0.5-2.0)	0.5 (0.0-1.25)	.28
DRS (%ml)	0.21 (0.05-0.28)	0.14 (0.0-0.4)	.36
FEV1 (%pred)	105.5 (100-110)	99 (92-109)	.10
FEV1/FVC (%)	86 (79-93)	80 (76-88)	.20
MMEF (%pred)	106.5 (89-115)	85 (73-112)	.14

Data are shown as median (interquartile range) or as a mean ± SD.

The value of DRS above 0.55 was present in 39% of patients (from 9.8 to 17.1% at various stages of the study). There were no significant seasonal variations in DRS and spirometric parameters.

Upon controlling for treatment groups, there were differences in eosinophilia changes. Significant increases in sEos were only observed in patients receiving placebo in comparison with baseline values (Table 2, Figure 1). We did not observe a change in eosinophil content at 3 and 6 months in the ML treatment group, as compared to initial assessments. There was a significantly greater change in the sEos count in patients after 6 months in the placebo treatment regime as compared to change in the ML group: 3.75 (6.0) vs 1.0 (2.0); $P = .007$.

A comparative analysis of the study groups showed a significantly lower value of sEos after 6 months in patients receiving the ML treatment (Table 2).

Interestingly, after the introduction of ML from the 7th month in the cohort receiving previously placebo (PI-ML), a reduction in the eosinophil content was observed at 9 months and 12 months as compared to the value at 6 months (Table 2). Comparative analysis of the groups showed no significant differences between final sEos values after 12 months of either the montelukast or placebo treatment (Table 2).

In the first and second halves of the yearlong study, values of both DRS and spirometry (FEV_1 , FEV_1/FVC , MEF) did not differ between both study groups. Common colds occurred in 12 patients in autumn, winter and spring (6 cases in each of the groups: placebo and montelukast), and 1 in the summer. There were no significant differences in the sEos content in all participants regardless of the presence of the common cold in the autumn, winter or spring periods (P : .14, .83, .36, respectively).

According to our knowledge, this is the first yearlong observation of eosinophilic inflammation in cases of asthma remission. During

the 12-month study period, we found elevated eosinophilia in induced sputum of 66% participants (20%-53% in 5 different assessments). Values obtained in our tests in adolescents are comparable to the results obtained by a Japanese study of young adults in a longer remission period of 5-10 years.² On the other hand, Arshad et al reported low sEos levels obtained in a group of 18-year-old patients in asthma remission—similar to those of non-asthmatics. That study involved a group of 56 patients, mainly with a non-atopic asthma (57%) and normal AHR. However, adequate sputum was obtained only in 16 cases.⁶

The results of our study showed a pronounced effect of seasonality on eosinophilic inflammation as determined by the percentage of eosinophils present in the induced sputum. During the autumn-winter period, the placebo group showed a significant increase in eosinophil content, as compared to the summer baseline. Furthermore, all the patients in asthma remission, either treated with montelukast or given the placebo, showed a similar trend. The literature on the subject lacks comprehensive long-term studies of eosinophilic inflammation in asthma remission pertaining to seasonality. However, the limited number of reports available in related topics supports our findings. For example, a yearlong study of paediatric atopic asthmatics (70% sensitized HDM) presented by Spanier et al reported the presence of significantly increased levels of a FeNO in the exhaled air in asthma patients during the autumn season.⁷ The effect of seasonality seems to be associated mainly with periodic stimulation by allergens (97.5% of our patients were allergic to house dust mites). Common infections, alongside the exposure to indoor allergens, play an important role in the severity of subclinical eosinophilic inflammation in the autumn and winter (12 in the autumn and winter vs 1 during the summer). sEos did not differ significantly between participants experiencing or not infections. However, tests were performed in relatively long 3-month intervals, usually not at the same time from last infection. In the context of observed significant seasonal variation in eosinophilic inflammation, the diagnosis of sustained remission of inflammation should be grounded in repetitive analysis of induced sputum. A practical alternative to such methodology could be the testing of sEos in the autumn and winter, particularly in patients who are allergic to house dust mites.

It appears that the diagnosis of complete asthma remission based on a negative methacholine challenge test is insufficient in the light of our understanding of eosinophilic inflammation during remission. The study by Komatsu et al showed that half of remission patients had negative results of methacholine-induced AHR, but only 32% of respondents had both a negative methacholine test and the correct percentage of eosinophils in induced sputum.² Consequently, it looks as if the recognition of complete remission can be based only on the repetitive testing of inflammatory markers and airway hyper-reactivity.

The presence of a subclinical persistent eosinophilic inflammation during asthma remission is a risk factor for disease recurrence, likely leading to a decline in lung function. It is estimated that 35% of patients in asthma remission will redevelop the disease.⁸ On

TABLE 2 Changes in sEos, DRS, FEV1 in PL-ML, ML-PL group and all study participants

	PL-ML (n = 18)	ML-PL (n = 23)	P	All patients
Baseline (summer)				
succs.IS	15	20	.91	35/41
Eos%	1.0 (0.5-2.0)	0.5 (0.0-1.25)	.28	0.5 (0.0-2.0)
nEos>2.5	3	4	1.00	7/35
DRS	0.21 (0.05-0.28)	0.14 (0.0-0.4)	.36	0.16 (0.05-0.28)
nDRS>0.55	2	2	.80	4/41
FEV1(%)	105.5 (100-110)	99 (92-109)	.10	103 (94-109)
3 mo (autumn)				
succs.IS	17	16	.11	33/41
Eos%	5.5 (1.5-7.5)**	2.5 (0.25-4.00)	.11	2.5 (1.0-6.5)**
nEos>2.5	10	6	.22	16/33
DRS	0.34 (0.13-0.58)	0.24 (0.13-0.34)	.35	0.27 (0.13-0.47)
nDRS>0.55	5	2	.14	7/39
FEV1(%)	106 (96-107)	97 (91-105)	.14	99 (91-111)
6 mo (winter)				
succs.IS	15	17	.73	32/41
Eos%	4.0 (2.0-10.0)***	2.0 (1.0-3.0)	.02	3.0 (1.5-8.0)**
nEos>2.5	11	6	.03	17/32
DRS	0.26 (0.15-0.41)	0.19 (0.04-0.36)	.28	0.23 (0.09-0.4)
nDRS>0.55	2	5	.37	7/41
FEV1(%)	102.5 (96-107)	94 (86-110)	.14	99 (90-108)
9 mo (spring)				
succs.IS	17	20	.79	37/41
Eos%	2.0 (0.5-4.5) [†]	1.5 (0.5-3.0)	.57	1.5 (0.5-3.5) ^{††}
nEos>2.5	7	6	.48	13/37
DRS	0.15 (0.12-0.21)	0.15 (0.10-0.36)	.84	0.15 (0.11-0.27)
nDRS>0.55	1	2	.70	3/41
FEV1(%)	102 (96-110)	96 (88-106)	.13	98 (91-108)
12 mo (summer)				
succs.IS	18	19	.18	37/41
Eos%	1.5 (0.5-5.0) ^{††}	1.0 (0.0-4.0)	.48	1.5 (0.5-4.0) ^{††}
nEos>2.5	5	6	.80	11/37
DRS	0.12 (0.0-0.34)	0.21 (0.03-0.30)	.40	0.16 (0.03-0.30)
nDRS>0.55	3	1	.19	4/41
FEV1(%)	106.5 (98-110)	98 (92-108)	.10	101 (93-109)

The comparison between groups was performed by the Mann-Whitney U test, and results are presented as median (Q25-Q75). Nonparametric data were analysed by chi-square tests.

DRS, dose-response slope: ratio of reduction of FEV1 (% of initial value) by volume of the sodium chloride inhaled (in ml); succs. IS—successful sputum induction [n (%)].

P = .01 and *P = .001 compared to baseline value; [†]P < .05 and ^{††}P < .01 compared to values after 6 months.

the other hand, in active asthma a high value of sEos is associated with a rapid decline in FEV₁.¹ Therefore, two questions remain as follows: 1) how anti-inflammatory treatment may affect the eosinophilic inflammation in asthma remission; and 2) what could be its long-term consequences. In the only available published study, van den Toorn et al found a beneficial effect of a 3-month course with fluticasone on some parameters of inflammation and remodelling

as determined by bronchial biopsies.⁹ Montelukast is a less potent anti-inflammatory agent than ICS. However, it could favourably influence remodelling present also during asthma remission.³ In our study, montelukast diminished eosinophilic inflammation during the seasonal increase associated with cold months. Our findings are consistent with clinical observations, indicating the beneficial effect of montelukast on seasonal exacerbations of asthma.¹⁰

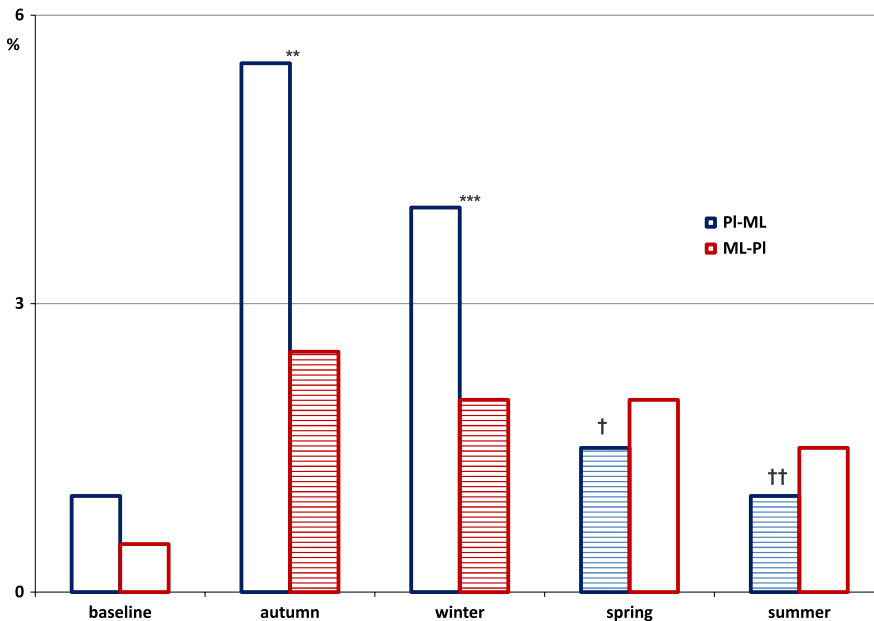


FIGURE 1 Seasonal variations of sputum eosinophilia (%) in both study groups (blue: PL-ML, red: ML-PI); filling with horizontal bars—montelukast usage. ** $P = .01$ and *** $P = .001$ compared to baseline value; † $P < .05$ and †† $P < .01$ compared to values after 6 months. [Colour figure can be viewed at wileyonlinelibrary.com]

Subjects in this yearlong study were in short-standing remission of asthma and the number of participants was small. However, the confirmation of clinical remission was a prospective further observation, during which 41 participants were completely free from symptoms of asthma. There was no wash-out period after 6 months' ML or PI treatment, but patients were directly entered in alternative treatment. Our study did not include the examination of other parameters of inflammation, such as FeNO or peripheral blood eosinophilia.

In summary, this yearlong study of eosinophilic inflammation indicates its incidental presence in the majority of young asthmatics with clinical remission of asthma and shows a clear relationship between inflammation and seasonality. It appears that in patients in asthma remission, montelukast mitigates eosinophilic inflammation and, especially, its seasonal fluctuations. It seems suitable to monitor occasionally inflammation markers or airway hyper-responsiveness during periods of high exposure to allergens in order to predict asthma recurrence.

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Association of Severe Atopic Dermatitis with month of birth in Armenian pediatric patients

To the editor,

Atopic dermatitis (AD) is a chronic relapsing skin disease which affects 15%-20% of children worldwide.¹ It is also considered as a major risk factor for developing other atopic diseases including food allergy, asthma, and allergic rhinitis later in life—a phenomenon known as the “atopic march.” Multiple factors, including season of birth and associated perinatal environmental conditions, have been known to play important roles in the manifestation of AD.² Moreover, about 20% of patients with AD have moderate-to-severe disease, which is associated with significantly lower quality of life, imposing considerable burden on the nation's health-care resources.³ Therefore, studying severe AD patients might be very important in managing overall AD-related disease burden. The objective of the present study was to identify potential birth season(s) associated with severe AD in Armenian pediatric patients, which would be helpful for the country's health-care researchers working toward identifying potential factors associated with severe pediatric AD in Armenia.

To evaluate the association between birth season and future development of severe AD, we analyzed de-identified data obtained from the patients' data cards (containing month of birth and AD severity levels of pediatric AD patients) of Arabkir Medical Center (AMC), Institute of Child and Adolescent Health, Yerevan, Armenia. All children diagnosed with AD between years 2008 and 2012 were included in this study (as the data were available). In the AMC hospital, severity was clinically graded into mild, moderate, and severe (SCORAD or EASI values were not available from the data cards) depending on dryness, redness, scratch marks, presence/absence of skin thickening (lichenification). Our final dataset consisted of de-identified information on age (in years), gender, month of birth, home-city, clinically determined severity and age at disease onset of 342 children. The mean age of the study cohort was 2.5 years, with 57% of the children being males. About 63.2% had disease onset before 1 year, whereas 19% had onset after 1 but before 2 years and 17.8% after 2 years. About 86.8% of the children had mild-to-moderate disease and 13.2% had severe disease. In logistic regression models, disease severity (severe vs mild/moderate) was treated as the

outcome variable, while gender, age at onset, month/season of birth were treated as predictor variables. Season was defined as follows: winter = December, January, and February; spring = March, April, and May; summer = June, July, and August; and fall = September, October, and November. All *P*-values below .05 were considered statistically significant. All analyses were carried out using the SAS statistical package (SAS Version 9.1, SAS Inc., NC, USA). Tables 1 and 2 show the results demonstrating the associations between season of birth and the severity of AD with spring (Table 1) and summer (Table 2) as reference seasons. Analysis adjusted for gender and age of onset that explored the association between birth season and AD severity with spring as reference season showed that being born in winter was associated with a lower risk of developing severe AD when compared to spring (OR = 0.2, 95% CI = 0.06, 0.64; *P*-value = .007). Gender was significantly associated with AD severity, with females having a lower risk of developing severe AD compared with males (OR = 0.32, 95% CI = 0.15, 0.68; *P*-value = .003). When the reference season was changed to summer, the results were similar, with winter births having a lower risk of developing severe AD when compared to summer (OR = 0.29, 95% CI = 0.09, 0.93; *P*-value = .04).

Existing data suggested strong relationships between the season of birth, perinatal exposure, and development of diseases including AD.^{2,4} Pediatric AD is a growing health problem in the republic of Armenia as indicated by steady increase in the number of registered pediatric AD cases nationwide, and in the capital city of Yerevan (Source: National Institute of Health of the Republic of Armenia; data not shown). However, there are no/very limited data currently available that could explain the connection between environmental exposure and severe AD manifestation in Armenian children. Characterizing the perinatal exposure, which is linked to birth season, could be very helpful in identifying the seasonal environmental risk factors associated with severe AD later in life. The present study indicates that being born in spring might be significantly associated with clinical manifestation of severe AD. Interestingly, previous authors have demonstrated the association between IgE-mediated aeroallergen sensitization and AD disease severity in German school children.⁵ Further studies are required to understand the mechanistic