

Original article

Influence of total IgE and seasonal increase of eosinophil cationic protein on bronchial hyperreactivity in asthmatic grass-sensitized farmers

Background: This study correlates biomarkers of atopy (serum total and specific IgE) and inflammation (serum eosinophil cationic protein) with bronchial hyperreactivity assessed after the complete end of pollination, in a group of farmers suffering from grass-allergic asthma.

Methods: A total of 28 asthmatic farmers, with allergy to grass pollen, reporting persistent asthma symptoms after grass pollination, were enrolled. An accurate allergologic screening excluded other sensitizations. Analysis of total and grass-specific IgE and eosinophil cationic protein was carried out before (March) and during (May) the following spring. After the complete end of pollination, bronchial hyperreactivity was assessed.

Results: Symptoms (cough, wheezing) persisted during the autumn for a mean period of 41 days (range 13–69). Total IgE was moderately high and grass-specific IgE ranged from 9.25 to 41.12 kU/l without significant differences before and during spring. On the contrary, serum ECP levels significantly increased during the pollination period. PD₂₀ methacholine evaluated after the end of grass pollination was negatively significantly correlated with levels of total IgE ($r = -0.73$; $P < 0.01$) and the increase (from March to May) of serum ECP ($r = -0.75$; $P < 0.01$). However, PD₂₀ methacholine did not correlate with grass-specific IgE and serum ECP absolute values of both March and May. A positive correlation was found between number of postseasonal days with symptoms and both spring increase of serum ECP ($r = 0.75$; $P = 0.04$) and levels of total IgE ($r = 0.76$; $P < 0.01$). The number of postseasonal days with symptoms inversely correlated with postseason PD₂₀ methacholine ($r = -0.76$; $P < 0.01$).

Conclusions: The study demonstrates that in grass-sensitized farmers with asthmatic symptoms persisting for several weeks after grass pollination has ceased, the degree of airways hyperreactivity and the duration of postseasonal symptoms are directly related to the spring increase of ECP levels, as well as to the level of total IgE in serum. This allows us to identify two candidate biomarkers for the risk of developing prolonged asthma symptoms, and for the effective monitoring of anti-inflammatory treatment and allergen-specific immunotherapy.

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Farmers have a higher prevalence of asthma than the general population (1, 2). This is caused by occupational exposure to components of organic dust (fungi such as *Alternaria alternata* and *Cladosporium herbarum* during grain harvesting and mites such as *Acarus siro* and *Lepidoglyphus destructor* during grain storage), to farm-animal dander (cow, horse, pig, etc.), and to pollens of grass and cereals. The inhalation of environmental allergens is the most important cause of asthma; it can be hypothesized that prolonged exposure to grass and cereal pollens during open-air work in the countryside predisposes farmers more frequently to

sensitization than the general population, and such exposure may induce late sustained reactions more frequently (1, 2).

Several effector cells are recruited and activated in bronchial inflammation and cause bronchial hyperreactivity, asthmatic symptoms, and finally irreversible morphologic changes in the bronchial mucosa (3). Eosinophils play a pivotal role in these inflammatory processes, as they release oxygen radicals, newly generated mediators such as platelet-activating factor, leukotriene C₄, and granule cationic proteins (major basic protein, eosinophil peroxidase, eosinophil cationic

protein, and eosinophil-derived neurotoxin/eosinophil protein X), which are responsible for bronchial epithelial cell damage and histopathologic changes (4, 5).

In grass-pollen-allergic asthmatics, symptoms of wheezing and/or cough frequently persist for several days after the pollination period. In some of these patients, asthmatic attacks may even occur throughout the whole year, even in the absence of other sensitizations, as to molds or mites, which are constantly present. The persistence of symptoms is probably due to the persistent airway hyperresponsiveness dependent on the bronchial inflammation induced by grass pollen.

The activity of eosinophils may be assessed by the detection of ECP in sputum or serum (6, 7), and studies in asthmatic patients have shown a correlation of serum ECP levels with severity of the disease, especially with respect to the late asthmatic response (8, 9).

A group of asthmatic farmers allergic to grass pollen was enrolled in this study with the aim of evaluating the role of inflammation (assessed through serum levels of ECP and total and specific IgE) in the maintenance of symptoms and bronchial hyperreactivity after the pollination period.

Material and methods

Twenty-eight farmers were enrolled (18 men and 10 women, with ages ranging from 28 to 44 years). All had been suffering from asthma for an average of 6 years (range 1–15) and attended an outpatient clinic during the autumn. They did not suffer from other respiratory diseases. Most of them (12 subjects) were nonsmokers, eight were current smokers, and eight were former smokers. All patients had daily and severe asthma symptoms (wheezing and cough) in spring and summer; the symptoms persisted in autumn after the grass pollination period, and disappeared by the end of November. Skin prick tests were positive (wheal diameter of >3 mm) to grass pollen. A detailed allergologic screening ruled out sensitizations to other allergens, including those of storage mites (*A. siro* and *L. destructor*), molds (*A. alternata* and *C. herbarum*), and farm-animal dander (cow, horse, pig, etc.), common causes of occupational exposure among farmers. They had never received allergen-specific immunotherapy. In the following year, analysis of total and grass-specific IgE, and ECP (CAP FEIA Pharmacia-Upjohn, Uppsala, Sweden) was carried out in March (before grass pollination) and in May (during grass pollination). During spring, all patients were treated with bronchodilators (short-acting β_2 agonist) as needed, without receiving anti-inflammatory medications. Subjects were seen again in the outpatient clinic at the end of September, and all reported the persistence of asthma symptoms. They were instructed to record

symptoms (cough, wheezing) in a diary until they were no longer symptomatic and were asked to return to the clinic at the end of November, or when symptoms had been absent for at least 7 days, for assessment of lung function and bronchial reactivity. Two patients recorded their last symptoms on 29 November and one on 3 December; thus, assessment of their lung function and bronchial reactivity was completed by 10 December.

No patient suffered from upper and lower respiratory tract infections for the entire period of study. Spirometry was performed with a wet spirometer (Sensormedics, P 2450). Bronchial hyperreactivity was assessed with methacholine (Lofarma, Milan, Italy), which was delivered with a dosimeter (Mefar MB3, Italy). After baseline spirometry, patients inhaled buffered saline vehicle solutions (eight doses), repeating spirometry at 90 and 180 s. Then they inhaled increasing concentrations of methacholine until a cumulative dose of 3200 μ g was reached or at least a 20% fall in FEV₁ occurred. Methacholine doses were delivered according to the protocol described by Ryan et al. (10). Bronchial hyperreactivity was measured by values of PD₂₀ expressed as the natural logarithms of the cumulative dose of methacholine causing at least a 20% fall in FEV₁.

The complete end of pollination was established by local aerobiologic monitoring to be around 25 September.

The original study included 32 farmers, but two were excluded because of respiratory infections in September, and two others because they did not record symptoms. The data of the evaluated patients were complete.

Statistical evaluation

Student's *t*-test and the Pearson coefficient were used to determine statistical association between the studied parameters.

Results

Symptoms (cough, wheezing) persisted after the complete end of grass pollination (verified at the end of September) for a mean period of 41 days (range 13–69).

Values of total IgE, grass-specific IgE, and serum

Table 1. Biomarkers of atopy (serum total and specific IgE) and of inflammation (serum ECP) in asthmatic farmers with allergy to grass pollen

		Mean	SD
Total IgE before spring	kU/l	620	229
Total IgE during spring	kU/l	700	280
Grass-specific IgE before spring	kU/l	26	8
Grass-specific IgE during spring	kU/l	16	6
ECP before spring	μ g/l	16.39	6.54
ECP during spring	μ g/l	51.82	16.61

ECP detected in March and May are summarized in Table 1. There were no statistically significant differences between total and specific IgE levels before and during the pollen season. However, serum ECP levels were significantly higher in May than in March.

Spirometry performed in autumn showed FVC and FEV₁ to be higher than 80% of the predicted values in all patients, FVC ranging from 80.3% to 110.7% and FEV₁ from 80.5% to 107.2%. The other lung-function parameters were also higher than predicted values, except for three patients with PEF, FEF₅₀, FEF₂₅, and FEV₂₅₋₇₅ below the predicted values. No correlation was found between lung-function parameters and both number of days in autumn with symptoms and serum ECP and IgE.

PD₂₀ methacholine ranged from 50 to 1600 µg/l; in particular, severe hyperreactivity (PD₂₀=50 µg/l) was evidenced in five patients, intermediate (PD₂₀ from 100 to 400 µg/l) in 16 patients, and mild (PD₂₀=800 µg/l) in four patients. Three patients presented normal bronchial reactivity (PD₂₀≥1600 µg/l).

PD₂₀ methacholine was inversely correlated with levels of total IgE ($r = -0.73$; $P < 0.01$) (Fig. 1) and the spring increase (from March to May) of serum ECP ($r = -0.75$; $P < 0.01$) (Fig. 2). On the contrary, PD₂₀ methacholine did not correlate with grass-specific IgE and serum ECP absolute values assessed in both March and May. Moreover, number of days with symptoms after the complete end of pollination positively correlated with the spring increase of serum ECP ($r = 0.75$; $P = 0.04$) (Fig. 3) and levels of total IgE ($r = 0.73$; $P < 0.01$). However, they inversely correlated with PD₂₀ methacholine determined in the same period ($r = -0.76$; $P < 0.01$) (Fig. 4). The duration of disease (number of years from diagnosis to the study enrollment), on average 6.1 ± 3.7 , did not correlate with studied parameters.

Discussion

We studied a selected group of farmers showing sensitization to grass pollen and asthma symptoms, which persisted long after pollination had ceased. The levels of total serum IgE and the magnitude of ECP increase during the pollen season were found to be the predictors of persistent bronchial inflammation and hyperreactivity, leading to prolonged duration of symptoms. These findings may be important for the assessment of long-term bronchial inflammation leading to hyperreactivity and development of symptoms even after the pollen season, in the absence of the sensitizing allergens.

Our results show that total serum IgE is a stronger indicator of disease activity than specific serum IgE, which merely reflects the degree of allergen sensitization. The levels of serum IgE did not change

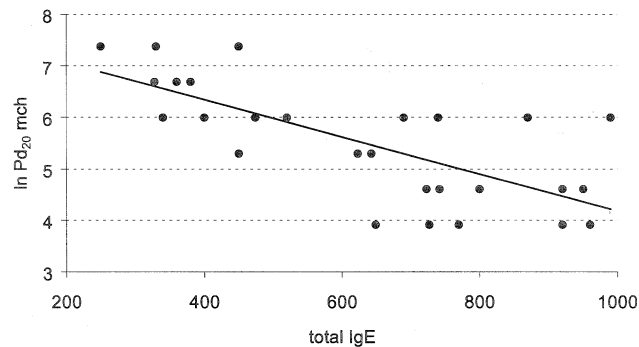


Figure 1. Correlation between total IgE and FEV₁ PD₂₀ methacholine (mch) expressed as natural logarithm (ln) in asthmatic farmers allergic to grass pollen ($r = -0.73$; $P < 0.01$).

significantly during the pollen season in this group of grass-sensitized farmers. Therefore, the postulated relevance of seasonal increase of total IgE does not apply to them, and, more importantly, only one determination is needed to establish the degree of risk of prolonged symptoms. However, total serum IgE was moderately high in this group; therefore, conclusions may differ for other groups of asthma patients.

Furthermore, the fact that single determinations of serum ECP values, either in March or in May, were not related to any of the lung-function parameters, and were not predictive of the degree of bronchial hyperreactivity after the pollination season, conflicts with several reports showing a negative correlation between ECP levels and lung function (11–13). In fact, the increase in serum ECP level during spring appeared to be the most significant prognostic marker of the severity of bronchial inflammation. Interestingly, total IgE significantly correlated with the increase of serum ECP and levels of bronchial hyperreactivity assessed in autumn after the complete end of pollination. High levels of total IgE represent a risk factor for development of allergic sensitization and onset of asthma (14, 15).

Evidence suggests that untreated allergic inflamma-

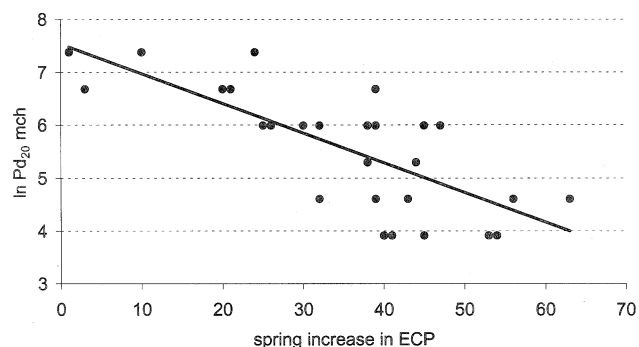


Figure 2. Correlation between spring increase (March to May) in serum ECP (µg/ml) and FEV₁ PD₂₀ methacholine (mch) expressed as natural logarithm (ln) in asthmatic farmers allergic to grass pollen ($r = -0.75$; $P < 0.01$).

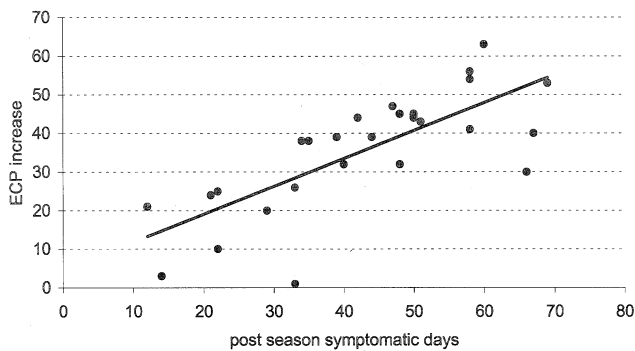


Figure 3. Correlation between postseason symptomatic days and increase (March to May) in ECP ($\mu\text{g/ml}$) in asthmatic farmers allergic to grass pollen ($r=0.75$; $P<0.04$).

tion may persist for several months, even in the absence of exposure to the specific allergens (16, 17). A negative correlation between ECP and lung function has been established in patients with allergic asthma, supporting the concept that ECP levels may reflect the chronic rather than the acute phase of asthma (18). When untreated, inflammation may become chronic and may cause the decline of lung function (9, 19). Persistence of inflammation may be assumed in the enrolled patients, as they reported asthma symptoms for an average of 41 days after the end of pollination. Moreover, bronchial hyperreactivity assessed after the end of pollination confirmed the underlying bronchial inflammation.

In conclusion, our study demonstrates that in grass-sensitized farmers with asthmatic symptoms occurring for several weeks after grass pollination has ceased, the

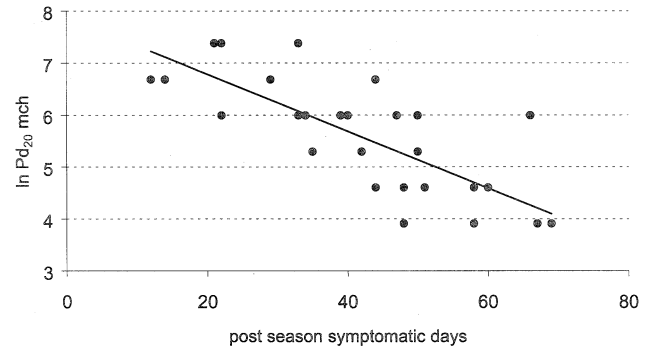


Figure 4. Correlation between postseason symptomatic days and FEV_1 PD_{20} methacholine (mch) expressed as natural logarithm ($\ln$) in asthmatic farmers allergic to grass pollen ($r=-0.76$; $P<0.01$).

degree of airways hyperreactivity and the duration of postseasonal symptoms are directly related to the increase of ECP levels during the pollen season, as well as to the level of total IgE in serum. This allows us to identify two candidate biomarkers (easily usable in routine clinical practice) for the risk of developing prolonged asthma symptoms, and for the effective monitoring of anti-inflammatory treatment and allergen-specific immunotherapy.

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