

# Eosinophil Cationic Protein Concentrations among Crop and Dairy Farmers with Asthma

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## Abstract

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**OBJECTIVE:** To assess the mean serum eosinophil cationic protein (s-ECP) concentrations among crop and dairy farmers and office controls, and further examine its relation to exposure duration, smoking habit, as well as presence or absence of asthma.

**METHODS:** A cross-sectional survey was performed including examined group (EG), composed by agricultural workers (87 crop - EG1 and 83 dairy farmers - EG2), and control group (CG) composed by 80 office workers within the same enterprise. We have used a questionnaire to record the chronic respiratory symptoms, detailed work history, specific farming activities and tasks performed and smoking history. Evaluation of examined subjects also included lung function tests, diagnosis of asthma, and measurement of s-ECP as a marker of inflammation.

**RESULTS:** The main finding of the present study is that s-ECP concentrations were raised in subjects with asthma independent of the smoking habit. The mean s-ECP concentrations were higher in subjects of EG1 and EG2 compared with those in CG, but without reaching statistical significance. Mean s-ECP concentrations were significantly higher among subjects in EG1 exposed more than 20 years, while mean s-ECP concentrations were non-significantly higher in subjects of EG2 exposed more than 20 years, compared to those exposed less than 20 years. Mean s-ECP concentrations were higher among smokers within all three groups, but without reaching statistical significance between smokers and non-smokers. Mean s-ECP concentrations were significantly higher in subjects with asthma within EG1 ( $P = 0.049$ ) and EG2 ( $P = 0.040$ ), but also within those in CG ( $P = 0.046$ ).

**CONCLUSION:** Data obtained suggest that airway inflammation is present in farmers with asthma, and s-ECP is an important biomarker in means of reflecting disease severity and prognosis among exposed workers.

## Introduction

Asthma is a chronic inflammatory disease of the airways in which many inflammatory cells have been found to play a role, particularly mast cells, eosinophils and T-lymphocytes. The association between eosinophilia and asthma was observed shortly after eosinophils were discovered. In patients with asthma, eosinophils are present in increased numbers in the blood, sputum and bronchoalveolar lavage fluid [1].

After activation, eosinophils can release

granulocyte-derived proteins, the most toxic of which are eosinophil cationic protein (ECP) and major basic protein (MBP) [2]. Clinical research has suggested emerging clinical usefulness of eosinophil granule proteins as serological markers in the assessment and management of asthma, of which ECP has been most widely characterised and researched. The results of many studies indicate that the degree of eosinophilic expression, that is, the levels of ECP in the blood and serum correlate with the degree of asthma severity and the extent of the achieved asthma control [3].

Immunologically potent substances such as antigens, endotoxins, glucans and substances with

complement-stimulating and adjuvant effect have been identified in the agricultural dust, exposure to which may provoke inflammatory reactions in the airways [4]. In a population study of farmers, lifetime cumulative prevalence of asthma was 6.3%, and for current asthma was 3.1%. Melbostad et al. found that animal production (husbandry) and familial predisposition interact as risk factors for asthma in farmers, and that atopy and specific allergies to cow, swine, grass and mites (*D. pteronyssinus*, *L. destructor* and *T. putrescentiae*) are associated with asthma and work-related upper and lower airway symptoms [5]. Eosinophil airway inflammation is characteristic of asthma, and, as shown in provocation studies, seems to be related to current asthma activity and recent allergen exposure [6]. Serum ECP value is a relevant marker of current eosinophil inflammatory activity in asthma [7]. There is a need for markers of airway inflammation in epidemiological studies of asthma.

In an epidemiological study of asthma in Norwegian farmers, Melbostad et al. [8] investigated s-ECP values in cases of atopic and non-atopic asthma, respectively, and in a control group without asthma and atopy. They also studied the relationship between airway obstruction, as an indicator of asthma activity, and s-ECP values, and whether ECP values were related to some positive allergen tests and specific allergies in asthmatic farmers.

Activated eosinophils in the asthmatic release their granular proteins, supporting the view that they have a pro-inflammatory role in the development of airway narrowing in asthma [9]. One such protein, ECP, was detected in bronchial biopsies and measured BAL [10], sputum and peripheral blood [11]. Serum and sputum ECP levels have been found to be correlated with the severity of asthma and allergen exposure [12]. It has been found that ECP can be used to monitor asthma inflammation [13].

The Italian study conducted among asthmatic grass-sensitized farmers by Di Gioacchino et al. [14] demonstrates that in grass sensitized farmers with asthmatic symptoms occurring for several weeks after grass pollination has ceased, the degree of airway hyperresponsiveness and the duration of post seasonal 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 identification of 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. The review of the literature indicated that s-ECP might serve as an objective indicator for clinical activity in asthma, and point to a possible pathophysiological axis in asthma that is based upon altered airway resistance due to eosinophils and eosinophil activity markers [15].

In the actual study we have assessed the

mean s-ECP concentrations among crop and dairy farmers and office controls, and further examined its relation to exposure duration, smoking habit, as well as the presence or absence of asthma.

## Subjects and Methods

### Study design and setting

We have performed cross-sectional research in the Center for Respiratory Functional Diagnostics at the Institute for Occupational Health of Republic of Macedonia, Skopje - WHO Collaborating Center for Occupational Health and GA<sup>2</sup>LEN Collaborating Center within the period September 2014 and April 2015. The examined and control groups are the same cohorts that were used in our previous study [16].

### Subjects

The survey included workers employed at agricultural enterprise divided into two groups: examined group (EG), composed by agricultural workers (crop and dairy farmers), and control group (CG) composed by office workers within the same enterprise.

EG consists of 170 subjects, while CG has 80 examinees. For the study purposes, and depending on the main agricultural activity, subjects were divided into two groups, examined group 1 (EG1) and examined group 2 (EG2). EG1 comprised 87 crop farmers (mean age =  $53.4 \pm 7.8$  years) engaged in crop farming (mean duration of exposure  $22.9 \pm 7.8$  years) with main activities composed of cultivating crops and vegetables, planting, digging, use of mechanised equipment, irrigation, and pesticide handling. They were exposed to various respiratory agents: dust, inappropriate climate, fumes, vapours and pesticides. EG2 consists of 83 dairy farmers (mean age =  $52.6 \pm 8.7$  years) employed as dairy farmers (mean duration of exposure  $23.7 \pm 7.6$  years), working inside confinement buildings, and exposed to: dust, inappropriate microclimate conditions, chemical hazards, vapors, gases, heavy manual work, animal contact, unfavorable body positions, and repetitive hand movements. Their main occupational activities were: preparation of fodder feeding and animal meals, milking, staying in the barn, preparation of straw, and haymaking, cattle raising, as well as taking care about milk hygiene and animal health.

Also, a similar group of 80 office workers (mean age =  $52.7 \pm 8.2$  years) with no exposure to respiratory agents, matched for age, duration of employment, daily smoking and socioeconomic status was studied as a control.

The Institute's ethics committee has approved

the content of our study protocol, whereas each examined subject was informed and gave written consent before any involvement in the study.

### Questionnaire

All study subjects were interviewed by the standardised questionnaire, including questions on work history, respiratory symptoms in the last 12 months, and smoking habit.

Chronic respiratory symptoms in the last 12 months (a cough, phlegm, dyspnea, wheezing, and chest tightness) were obtained using the European Community for Coal and Steel questionnaire (ECCS - 87), and the European Community Respiratory Health Survey (ECRHS) questionnaire [17] [18]. Classification of smoking status was done according to the World Health Organization (WHO) guidelines on definitions of smoking status [19].

Daily smoker was defined as a subject who smoked at the time of the field survey at least once a day, except on days of religious fasting. Among daily smokers, lifetime cigarette smoking and the daily mean of cigarettes smoked were also assessed. Pack-years smoked were calculated according to the actual recommendations [20]. Ex-smoker was defined as a formerly daily smoker, no longer smokes. Passive smoking or exposure to environmental tobacco smoke (ETS) was defined as the exposure of a person to tobacco combustion products from smoking by others [21].

### Spirometry

All study subjects underwent spirometry testing, performed by spirometer Ganshorn SanoScope LF8 (Ganshorn Medizin Electronic GmbH, Germany), measuring forced vital capacity (FVC), forced expiratory volume in one second (FEV<sub>1</sub>), FEV<sub>1</sub>/FVC ratio, and maximal expiratory flow at 50%, 75%, and 25–75% of FVC (MEF<sub>50</sub>, MEF<sub>75</sub>, and MEF<sub>25-75</sub>, respectively), by recording the best result from three measurements of the values of FEV<sub>1</sub> within 5 % of each other. The results were expressed as percentages of the predicted values according to the European Community for Coal and Steel (ECCS) norms [22].

### Diagnostic criteria for asthma

According to the actual recommendations by Global Initiative for Asthma (GINA) asthma in subjects with normal spirometric findings is defined as symptomatic bronchial hyperresponsiveness (BHR) with PC<sub>20</sub> ≤ 4 mg/mL, while in those with lower values of spirometric parameters as a positive bronchodilator test [23].

### Measurement of s-ECP concentrations as markers of inflammation

Mean s-ECP concentrations are measured by chemiluminescent immunoassay method with Immulite 1000 in subjects with chronic respiratory symptoms and spirometric impairment. s-ECP concentrations < 24 µg/L are within the normal range [24].

### Statistical analysis

We have analysed the data using Statistica for Windows version 7. Continuous variables were expressed as mean values with standard deviation and categorical variables as numbers and percentages. The comparison of mean ECP serum concentrations was performed by independent-samples T-test. A P-value of less than 0.05 was considered statistically significant.

## Results

Table 1 gives an overview of the demographic and overall characteristics of the subjects within the examined and control groups.

**Table 1: Demographic and overall characteristics of the study subjects**

Variable	EG1 (n = 87)	EG2 (n = 83)	CG (n = 80)
Gender/M/F ratio	3.6	2.6	2.7
Age/years	53.4 ± 7.8	52.6 ± 8.7	52.7 ± 8.2
BMI/ kg m <sup>2</sup>	25.1 ± 3.5	25.4 ± 3.6	26.2 ± 3.7
Duration of employment / years	29.2 ± 8.9	26.3 ± 10.1	25.3 ± 9.8
Exposure duration / years	22.9 ± 7.8	23.7 ± 7.6	/
Active (daily) smokers	45 (51.7%)	39 (46.9%)	39 (48.7%)
Smoking experience/years	19.7 ± 8.1	18.9 ± 7.6	19.2 ± 7.8
Cigarettes / day	15.4 ± 7.3	14.6 ± 6.8	14.8 ± 7.2
Ex-smokers	12 (13.8%)	9 (10.8%)	12 (15%)
Passive smokers	11 (12.6%)	8 (9.6%)	7 (8.7%)

Numerical data are expressed as mean values with standard deviations; frequencies of active, passive, and ex-smokers are given as number and percent of subjects with certain variable. M: males; F: females; BMI: body mass index.

Table 2 shows the frequencies of asthma symptoms (a cough, dyspnea, wheezing and/or chest tightness), positive BD tests, positive non-specific histamine challenge tests with PC<sub>20</sub> ≤ 4 mg / mL, as well as asthma detected among subjects in EG1, EG2 and CG.

**Table 2: Frequency of asthma symptoms, positive BD tests, positive histamine challenge tests with PC<sub>20</sub> ≤ 4 mg/mL, and asthma among subjects in EG1, EG2 and CG**

Variable	EG1 (n = 87)	EG2 (n = 83)	CG (n = 80)
Respiratory symptoms in the last 12 months	26 (29.9%)	24 (28.9%)	16 (20%)
Positive BD test	15 (17.2%)	14 (16.9%)	8 (10%)
Positive histamine challenge test with PC <sub>20</sub> ≤ 4 mg/mL	9 (10.3%)	7 (8.5%)	5 (6.3%)
Asthma	7 (8%)	6 (7.2%)	4 (5%)

Data are given as number and percent of subjects with a certain variable.

For the study purposes, we have examined

the marker of chronic eosinophil inflammatory activity-s-ECP among subjects in the three groups having one or more chronic respiratory symptoms and / or spirometric impairments.

**Determination of mean s-ECP concentrations**

The mean s-ECP concentrations were higher in EG1 compared to CG, but without statistical significance (Table 3).

**Table 3: Mean s-ECP concentrations in subjects of EG1 and CG**

	EG1 (n = 35)	CG (n = 15)	P-value*
s-ECP (µg/L)	14.4 ± 3.7	12.6 ± 3.2	0.108

Data are given as means with standard deviation. \* Tested with t-test for independent samples.

Mean s-ECP concentrations were higher in subjects of EG2 compared to those of CG, but statistical significance is not yet reached (Table 4).

**Table 4: Mean ECP serum concentrations in subjects of EG2 and CG**

	EG2 (n = 32)	CG (n = 15)	P-value*
s-ECP (µg/L)	13.9 ± 3.5	12.6 ± 3.2	0.229

Data are given as means with standard deviation.\* Tested with t-test for independent samples.

Table 5 shows the mean s-ECP concentrations in subjects of EG1 with exposure duration less or equal to 20 years and over 20 years.

**Table 5: Mean ECP serum concentrations in subjects of EG1 according to job exposure duration**

	Exposed > 20 years (n = 24)	Exposed ≤ 20 years (n = 11)	P-value*
s-ECP (µg/L)	16.2 ± 4.3	13.1 ± 3.1	0.039

Data are given as means with standard deviation.\* Tested with t-test for independent samples.

Mean s-ECP concentrations were significantly higher in subjects of EG1 exposed more than 20 years, compared to those with job exposure less than 20 years.

Table 6 gives an overview of mean s-ECP concentrations in subjects of EG2 with duration of exposure less or equal to 20 years and over 20 years.

**Table 6: Mean s-ECP concentrations in subjects of EG2 according to job exposure duration**

	Exposed > 20 years (n = 22)	Exposed ≤ 20 years (n = 10)	P-value*
s-ECP (µg/L)	15.1 ± 3.9	12.6 ± 3.2	0.086

Data are given as means with standard deviation.\* Tested with t-test for independent samples.

Mean s-ECP concentrations were higher in subjects of EG2 exposed longer than 20 years compared to those with a shorter period of job exposure but without statistical significance.

The mean s-ECP concentrations in subjects of all 3 groups depending on the smoking habit are given in Table 7.

**Table 7: Mean s-ECP concentrations in subjects of EG1, EG2, and CG depending on smoking habit**

Variable	EG1 (n = 35) 21 vs 14	P*	EG2 (n = 32) 19 vs 13	P*	CG (n = 15) 9 vs 6	P*
s-ECP in active smokers	15.5±4.1	0.208	14.9±3.8	0.107	13.7±3.6	0.664
s-ECP in non-smokers	13.8±3.4		12.7±3.5		12.9±3.1	

Data are given as means with standard deviation.\* Tested with t-test for independent samples.

The mean s-ECP concentrations were higher in smokers within the three groups, but without significant difference between smokers and non-smokers.

Mean s-ECP concentrations in all three groups due to the presence or absence of asthma are shown in Table 8.

**Table 8: Mean s-ECP concentrations in subjects of EG1, EG2, and CG due to the presence or absence of asthma**

Variable	EG1 (n = 35) 7 vs 28	P*	EG2 (n = 32) 6 vs. 26	P*	CG (n = 15) 4 vs. 11	P*
s-ECP in subjects with asthma	18.9 ± 7.5	0.013	17.2 ± 6.9	0.039	16.8 ± 4.9	0.046
s-ECP in subjects without asthma	13.6 ± 3.8		12.9 ± 3.7		12.1 ± 3.2	

Data are given as means with standard deviation. \* Tested with t-test for independent samples.

The mean s-ECP concentrations were significantly higher in subjects with asthma compared to non-asthmatics in all three groups.

**Discussion**

The average s-ECP concentrations were higher in subjects within EG1 and EG2 compared to those in CG, but without statistical significance. The average s-ECP concentrations were significantly higher among subjects in EG1 exposed more than 20 years, compared to those with exposure less than 20 years, while average s-ECP concentrations were non-significantly higher in subjects of EG2 exposed more than 20 years, compared to those exposed less than 20 years. The average s-ECP concentrations were higher among smokers within all three groups, but without reaching statistical significance between smokers and non-smokers, whereas they were significantly higher in asthmatic subjects compared to those without asthma in all three groups. Similar

results were obtained in the study of Heldal et al. [25] dedicated to occupational exposure to bio-aerosols while ECP as a marker of chronic airway inflammation was confirmed in the research conducted by Hamed et al. focused on the its predictive value in subjects with poorly controlled asthma and therapeutic response to inhaled corticosteroids [26].

Substantial research work has been carried out to determine changes in s-ECP levels due to different allergic and non-allergic diseases over the last decades and especially during last two decades. As a result of these studies, enough quality work is now available to bridge the link between eosinophil activity and allergy phenomenon. Serum ECP is now closer to be declared as an established marker of allergy [27]. Many reported studies demonstrate an increase in s-ECP concentrations in asthmatic patients as compared to healthy controls [28][29]. Amongst the notable studies of eosinophil activity markers in induced sputum two studies found that ECP levels were significantly positively correlated with the mean weekly total symptom scores [30].

The concentration of serum ECP has recently been found to correlate with ECP concentration in bronchoalveolar lavage fluid (BALF) [31]. Therefore, assessment of s-ECP may be considered to reflect pulmonary inflammation in asthma [32]. Studies of asthmatic patients, especially adults, have indicated a relationship between the level of serum ECP and the severity and nature of the disease [33][34]. The present study showed that the s-ECP levels were significantly elevated in asthmatic subjects as compared to that of healthy controls. This indicated the role of eosinophilic inflammation in the pathogenesis of asthma. It is clear that our results were consistent with previous studies that have shown that higher ECP levels in the serum of asthmatic patients when compared with healthy subjects [33] [34] [35]. Measuring of s-ECP levels have the advantages over eosinophilic count in that it reflects not only the number of cells but also their degree of activation and is, therefore, a better inflammatory marker [35]. The present study results show significant higher s-ECP levels in asthmatic subjects than those without asthma, regardless of the occupational exposure to respiratory hazards. Other studies [29] [34] [36] reported the same association between s-ECP levels and asthma severity.

Thus assessment of s-ECP may be a reflection of pulmonary inflammation in bronchial asthma [37]. The presence of eosinophilic inflammation seems to be of importance since this feature is inconsistently observed in non-asthmatic atopic patients and absent in patients with a chronic cough [38]. Furthermore, these results indicate that although eosinophils are recruited in intermittent asthma, they are less activated in persistent asthma. The different patterns of eosinophilic activation found in persistent as compared with intermittent asthma might be important consequences of the integrity of

the bronchial mucosa [39].

Direct measurement of airways inflammation using biological markers could potentially refine asthma management. This explains the current research interest in measuring levels of exhaled nitric oxide and eosinophil granule proteins especially s-ECP in asthma [40]. The study by Zedan et al. [41] revealed that both peripheral eosinophil count and s-ECP levels were significantly higher in atopic asthmatics as a group than in healthy control subjects. On the other hand, both parameters were significantly higher among partially controlled asthma cases compared with healthy control children as well as controlled asthma cases. Interestingly, however, controlled asthma cases showed non-significant changes in the levels of both parameters versus healthy control children. This finding is supported by the evidence that eosinophils play an important role in the pathogenesis of asthma and that elevation of peripheral blood eosinophil count is a risk factor for the development of airway remodelling and irreversible changes in lung function [42]. This is also supported by the research of Lee et al. who reported that higher levels of s-ECP were associated with more severe exacerbation of asthma followed by a decrease in s-ECP levels with a resolution of symptoms [43].

Zedan et al. also showed a significant inverse correlation between the level of asthma control and both parameters, particularly s-ECP, implying that poorer control is expected with higher s-ECP levels [41]. This will add to the work of Koh et al., who described a correlation between asthma severity and s-ECP level. Thus, considering that s-ECP has been widely investigated as a potential biomarker of airway inflammation, it may have a useful role to play as a control parameter in asthma guidelines [2]. Mean s-ECP values were significantly higher in cases of current allergic asthma than in non-allergic asthma, and lowest in non-asthmatic, non-atopic controls. In atopic asthmatics, ECP showed significant associations with airway obstruction and numbers of RAST allergens positive, as well as specific allergies, e.g. to swine and *D. pteronyssinus* [44]. Serum ECP is not a discriminating test for identifying asthma in epidemiology but can be used as a supplement to questionnaires and spirometry to indicate current asthma activity [42].

The results of a recent study in our country showed that the ICS objectively suppress the inflammatory reaction in asthma and the biologic markers (IL-5, Eo and s-ECP), which if being followed, can measure the accomplished effect. Therefore, they could be used in everyday practice, not only as diagnostic parameters but also as valid therapeutic guides in the treatment of asthma [45]. Another similar research showed that eosinophils, s-ECP and IL-5 could be useful markers for selecting allergic patients and could be the monitors of treatment effects [46]. Assessing eosinophilic inflammation is therefore

important in establishing a diagnosis, in monitoring and assessing response to treatment, and in testing novel therapeutics. Clinical markers of atopy and eosinophilic inflammation include indirect tests such as lung function, exhaled breath condensate analysis, fractional exhaled nitric oxide, serum immunoglobulin E levels and serum periostin. Direct measures, which quantify but do not anatomically localise inflammation, include blood eosinophil counts, serum or plasma eosinophil cationic protein and sputum eosinophil levels. Cytology from bronchoalveolar lavage and histology from endobronchial and transbronchial biopsies are better at localising inflammation but are more invasive. Novel approaches using radiolabelled eosinophils with single-photon emission computed tomography offer the prospect of non-invasive methods to localise eosinophilic inflammation [47].

Our present study has some limitations. Namely, relatively small number of the subjects in the study groups may be a limitation, with possible implications on the data obtained and its interpretation, especially having in mind their extrapolation on the population level for the agricultural workers in the Republic of Macedonia. There is a lack of ambient monitoring and exposure measurement (endotoxin, dust, gases, vapours, and chemicals) in this survey. On the other hand, the data concerning exposure to respiratory hazards are based on job exposure matrices, introduced in our country. Finally, we can confirm that s-ECP is an important biomarker of airway inflammation present among farmers with asthma in means of reflecting disease severity and its prognosis. Furthermore, s-ECP levels were raised independently of smoking status in asthmatic subjects showing that s-ECP rise was a result of the inflammatory nature of the disease itself.

In conclusion, despite the small sample size, this study has demonstrated that s-ECP may have clinical usefulness in assessing levels of asthma control and hence in refining asthma management. Based on these findings, our recommendation is conduction of a larger, randomized controlled trial to evaluate the correlation between s-ECP level and degree of asthma control, in order to obtain a cut-off point for s-ECP beyond which farmers with asthma may be considered uncontrolled, and to extrapolate this point on the population level among agricultural workers, having in mind exposure duration and its characteristics in farming, as well.

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