ORIGINAL ARTICLE

The Predictive Value of Eosinophil Cationic Protein in Asthma as Marker of Poorly Controlled Disease and Response Guide to Treatment.

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Abstract

Background: Asthma is an inflammatory disease that involves local and systemic inflammation. Eosinophil cationic protein [ECP] is a marker of eosinophilic inflammation in asthma.
Objective: To clarify the validity of ECP determination as predictor of poorly controlled or relapsing asthma.
Patients and Methods: Twenty six asthmatic patients were included for poorly controlled asthma, while 12 patients were included in relapse study. ECP were determined using ELISA.
Results: Asthmatic patients with poorly controlled asthma had significantly ($P<0.0001$) higher sputum and serum ECP than that in stable asthmatic. Mean serum ECP of asthmatic patients was significantly higher than that in asthmatic patients without relapse.
Conclusion: Serum and sputum ECP as eosinophilic inflammatory markers are associated with poor asthma control and may be used as additional marker for disease control response guide to treatment.

Key words: Asthma, Poor control, ECP.

Introduction:

Lung infiltration by inflammatory cells, and especially eosinophils, is a characteristic pathological feature of bronchial asthma[1]. Activated eosinophils in asthmatic release their granular proteins, supporting the view that they have a pro inflammatory role in the development of airway narrowing in asthma[2]. One such protein, ECP, was detected in bronchial biopsies and measured BAL[3, 4], sputum and peripheral blood[5-12]. Serum and sputum ECP levels have been found to be correlated with the severity of asthma[13-22] and allergen exposure[23, 24]. It has been found that ECP can be used to monitor asthma inflammation[25, 26]. The purpose of this study was to clarify the predictive value of determination of ECP in follow up and monitoring the response to treatment and alarm for disease worsening in asthma.
The severity of asthma is usually assessed at the first visit. Treatment of asthma is based on asthma severity, with a step up approach, characterized by increased intensity of treatment for increased severity of asthma[27]. The primary goal of asthma treatment is the control of asthma, defined as the absence or presence of minimal symptoms, minimal or no requirement for rescue medications and normal or best lung function[27]. Poor control of asthma is characterized by the persistence of symptoms, night awaking, use of rescue medications, and diurnal variability of peak expiratory flow of > 20%. Scores to assess asthma control have reported recently[28].
The relationship between airway inflammation and asthma severity is controversial, particularly in severe asthmatics[29]. Sputum eosinophilia has been shown to correlate with asthma severity in some[31], but not in other studies[32-34]. Recently one report[35] investigated the relationship between airway eosinophilia and asthma control, cellular and biochemical markers of airway inflammation in 19 asthmatic patients. They reported that sputum eosinophils and ECP were significantly higher inpatients with poorly controlled asthma as compared to patient with controlled asthma.

Materials and Methods:

Patients:

The subjects included in the study were outpatients from the Asthma and Allergy Centre. The diagnosis of asthma was performed by specialist physician and was established according to the National Heart Blood and Lung Institute / World Health Organization (NHLBI/WHO) workshop on the Global Strategy for Asthma[27]. Patients were excluded if they were smokers, if they had respiratory infection within the month preceding the study, a rheumatologic illness, malignancy, diabetic, heart failure, history of venous embolisms, coronary heart disease and liver or kidney diseases. At enrolment, they all underwent full clinical examination, pulmonary function test, and blood sampling. Sputum samples were collected from patients when indicated. Normal volunteers were also enrolled in the study as a healthy control. None of them had any previous history of lung or allergic disease and were not using any medication. They had a normal lung function test (FEV1 > 80%) and negative skin allergy test.

General stool examination was performed in all patients and controls to exclude parasitic infections. The sampling was performed during the period from May 2004 to December 2005. All samples were collected in the morning following overnight fasting. ECP as a marker of eosinophilic inflammation in 26 asthmatic patients was evaluated to clarify the validity of its determination as predictor of poor control.

Asthmatic patients, regularly followed for at least 3 months, were randomly recruited for the study over a period of one month. All subjects were outpatients, without any acute severe asthma exacerbation (defined by the requirement of treatment with oral corticosteroids or a decrease in FEV1 >30% below the baseline value on two consecutive days) in the month preceding the study. All 26 patients received inhaled Beclomethasone dipropionate and Salbutamol inhalers for 4 weeks. At the end of this period, 12 patients were considered poorly controlled and 14 as well controlled. Poor control was based on symptom frequency, night wakening, bronchodilator use and FEV1 of > 20 % in respect of drug usage. Venous blood and sputum samples were collected at the end of the study to determine ECP.

The predictive value of serum ECP for asthma relapse during discontinuation of inhaled corticosteroids therapy was evaluated in 12 patients. All patients enrolled in the study were with stable asthma over a period of one month preceding the study. Inhaled Beclomethasone dipropionate was tapered and followed weekly for exacerbation attack. The patients informed to consult their physician between regular visits if they developed attack. Patients who experienced an asthma exacerbation which required a course of oral prednisolone were not included. After one month follow up, asthma relapse was found in 5 out of these 12 patients. Venous blood was collected at baseline, when exacerbation developed and after one month from subjects who did not develop an exacerbation to determine serum ECP. The research protocol was approved by Tikrit University College of Medicine ethical committee and informed consent taken from all patients included in the study.

Skin Prick Test:

The skin prick tests were performed and evaluated in accordance with European Academy of Allergy and Clinical Immunology subcommittee on allergy standardization and skin tests using standards allergen panel (Stallergen, France). The panel included the common inhalant allergens.

Sputum Collection:
Sputum was induced only when it could not be produced spontaneously. Sputum induction was performed as described by Fahy et al [30].

Determination of Serum Eosinophilic Cationic Protein:
Serum ECP determined by ELISA kit (MBL MESCACUP ECP TEST) from Medical and Biological Laboratories Co, LTD, and Japan.

Statistical Analysis
The values are reported as mean ± SD and 95% confidence interval. For statistical analysis between groups, paired t-test was used. P values of < 0.05 were considered significant.

Results:

ECP as Marker of Eosinophilic Inflammation in Poorly Controlled Asthma:
Asthmatic patients with poorly controlled asthma had significantly ($P<0.0001$) higher sputum (642 µg/l ± 172) ECP than that in stable asthmatic (374 µg/l ± 128). Furthermore, serum ECP in poorly controlled asthmatic was significantly ($P<0.0001$) higher (59.8 µg/l ±13.6) than that in stable asthmatic (27.5 µg/l ± 2.9) (Table.1). Thus serum and sputum ECP as eosinophilic inflammatory markers are associated with poor asthma control.

Predictive Value of Serum ECP for Asthma Relapse during Discontinuation of ICS Therapy:
Asthma relapse occurred in 5 out of 12 (41.6%) patients after 1 month. Mean serum ECP of asthmatic patients with relapse was significantly higher (42.1 µg/l ± 13.2, $P<0.001$) than that in those without relapse (28.3 µg/l ± 3.75). Furthermore, serum ECP in asthmatic with relapse was significantly higher ($P<0.0001$) than that for asthmatic subjects enrolled in the study at baseline (before step down reduction of ICS therapy) (Table.2). However, serum ECP in asthmatic without relapse was higher (28.3 µg/l) than that at baseline (18.8 µg/l) ($P = 0.05$). These findings suggest that serum ECP level may be predictive of subsequent asthma worsening when ICS dosage reduced.

Table: 1. Eosinophilic cationic protein (ECP, μg/l) as a marker for treatment response guide in asthma.

		Steroid controlled [14]	Steroid uncontrolled [12]	P value <
Serum ECP	Mean	27.5	59.8	0.0001
	SD	2..9	13.6	
	95%CI	25.8 – 29.2	51.2-68.4	
Sputum ECP	Mean	374	642	0.0001
	SD	128	172	
	95%CI	300-448	533-751	

Table: 2. Serum eosinophil cationic protein (ECP, μg/l) concentration in asthmatic patients during relapse.

Group	Number	Serum ECP concentration		
		Mean	SD	95% CI
Baseline	12	18.8	7.6	14-23.6
Relapse positive	5	42.1	13.2	25.7 – 58.5
Relapse negative	7	28.3	3.75	24.9-31.8

P values
Baseline vs. Relapse positive: < 0.0001
Baseline vs. Relapse negative: 0.05
Relapse positive Vs Relapse negative: < 0.001

Discussion:

In this study serum and sputum ECP as an eosinophilic mediator was increased significantly in poorly controlled asthma as compared to stable controlled asthma. This suggests that serum and sputum ECP levels may be predictive of poor control of asthma. Reddel et al [36] recommended the distinction between asthma exacerbation and poorly controlled asthma which has important clinical implications as the treatment of poorly controlled asthma simply requires the administration of increasing amounts of inhaled steroids combined with long lasting bronchodilators, whereas the exacerbations requires short course of systemic steroids[27].

In our study, 12 patients with chronic asthma of different severity, appropriately treated according to their level, were poorly controlled even if they were in an exacerbation [36]. As this study indicates, sputum and serum ECP were predictive in distinguishing between controlled and poorly controlled asthma. This may improve in our estimation of prognosis or outcome of asthma treatment.

Studies have show association between sputum eosinophilia and exacerbation of asthma by withdrawal of corticosteroids treatment [37, 38]. In the present study the poorly controlled asthma was not due to withdrawal of anti –inflammatory treatment and it was associated with increased serum and sputum ECP, suggesting a correlation between eosinophilic airway inflammation and clinical instability of the disease. This is consistent with the finding reported by Romagnoli et al [35] using different inflammatory markers.

Eosinophilic inflammation has been considered important determinant of asthma severity in some studies [29, 39], while other studies failed to confirm this [33, 38]. The results of this study confirms and extend those of previous ones that found a significant correlation between eosinophilic inflammation and loss of asthma control and that level of instability must be taken into account in trying to correlate severity with eosinophilic inflammation.

Reasons for poor control of asthma are not always known. Exposure to high levels of allergen may be an important factor in patients with severe asthma that is not readily controlled [41]. Exacerbating factors include viral infections [40], skin fungal infections [29], chemical sensitizers [42]. Exposure to the causative agents for more than 6 months may be associated with the persistence of asthma, even when it is avoided [41]. There is little evidence that chronic asthma is worsened by allergy to normal dietary components. However food additives such as sodium metabisulphite and tartrazine [42] and dietary salicylates [43] may worsen asthma in certain patients. In many patients with difficult asthma, psychological factors may play a role [44].

ECP has been used as marker of ongoing eosinophilic inflammation and for prediction of the clinical course of asthma [45, 46]. Regular treatment with ICS leads to a decrease in the number of inflammatory cells and improvement of asthma symptoms and lung function [47], although in some studies this relationship is not so obvious [48]. Treatment with ICS may give long lasting amelioration of symptoms, improved lung function and reduced BHR even after cassation of therapy [49, 50].

The present study results demonstrated that withdrawal of low dose of inhaled Beclomethasone dipropionate induces deterioration and increased airway inflammation measured by serum ECP. Asthma relapsed in 5 out of 12 such patients after one month. The serum ECP of asthmatic patients with relapse was significantly higher than that in asthmatic without relapse. Furthermore, serum ECP in asthmatic with relapse was significantly higher than for asthmatic subjects at baseline. This

finding indicates that serum ECP may be predictive of subsequent asthma worsening when ICS dosage is reduced.

Previous prospective studies of cessation of steroid therapy have assessed deterioration in asthma by means of lung function, BHR measurement and symptoms [49-51]. In a recent report, serum ECP increased in withdrawal group of asthmatic using inhaled Budesonide [51]. In a pediatric study of airway responsiveness, FEV1 deteriorated in children within 1 month after treatment with 600 µg/day of Budesonide was tapered off [51]. In contrast, Juniper et al [50] reported stable airway responsiveness but decreased FEV1 and clinical symptoms in adult patients after 3 months without inhaled Budesonide at 400 µg /day.

Withdrawal studies in adults have reported increased number of peripheral blood eosinophils in combination with increased exacerbation rates of asthma [53, 54]. In agreement with that, Lonnkvist et al [52] found, on a group level, a correlation between increasing number of blood eosinophils and serum level of ECP and increasing risk for asthma exacerbation. Baba et al [55] found that serum ECP was significantly higher in a group of adult asthmatics, with step down of inhaled Beclomethasone dipropionate than that without step down. Weaver et al [56] reported that adjusted steroid therapy guided by serum ECP level in adult's asthmatic may be helpful in tailoring asthma treatment. These two and our findings suggest that serum ECP levels may be predictive of subsequent asthma worsening when inhaled Beclomethasone dipropionate dosage is reduced.

The eosinophils are one of the main protagonists in this process; control of the proteins that are released by the eosinophils when it is activated (more specifically ECP) could provide valuable information for this purpose [23]. The ECP has also been used in the diagnosis of asthma, in the prognosis of wheezing infants, as markers to exposure to allergen, to evaluate the results of the provocation tests, with the clinical and functional seriousness of asthma, to control the effect of different anti-inflammatory treatments as well as to monitor the performance of this treatment [23].

It is necessary to appreciate that this marker is a reflection of the activation of the eosinophils [39], without specifying where the activation has been produced and therefore there are studies that document increase in ECP in relation to other conditions besides asthma e.g. rhinitis and atopic dermatitis [57]. On the other hand there is a certain overlapping of the ECP values of patients with the active illness and with normal controls, but it with low frequency [23]. However, when asthma is being controlled, the determination of the ECP in induced sputum could have a more precise value, while it could be a more accurate reflection of the inflammation that exists on a local level, although this is the fact that needs to be proved subsequently [13, 58].

The principal usefulness of the ECP is for the individual follow up and control of each specific patient [59]. Whatever the case, and given that the asthmatic inflammation is a very complex process, a suitable combination of different markers is needed, which predicatively improves the reliability of the diagnosis and prognosis and allows for more precise control of the asthmatic inflammation.

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