Eosinophil cationic protein: Is it useful in asthma? A systematic review

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Summary

Introduction: Eosinophil cationic protein (ECP) has been widely investigated as a potential biomarker of airway inflammation.

Method: A systematic review was performed using Medline with key terms eosinophil cationic protein and asthma, limiting the search to titles or abstracts. Out of 688 potential papers found, abstracts were reviewed based on the following criteria: (1) ECP was used as a biological marker, (2) asthma was the index disease studied, (3) it was a controlled clinical study and (4) ECP was assessed as a diagnostic, assessment or management tool. One hundred and sixty-nine articles satisfied the selection criteria and their full-text versions were reviewed. Only 53 papers were found to provide clinically useful information.

Results: ECP has been measured in serum, plasma, sputum, saliva and broncho-alveolar lavage fluids but serum and sputum are the most established. Levels of ECP in normal and asthmatic subjects in various body fluids were identified. ECP correlates well with airway inflammation but not airway hyper-responsiveness. It is raised in other atopic diseases and hence is not diagnostic for asthma. However, it has been shown to be useful in assessing asthma severity, compliance with anti-inflammatory asthma therapy and as a guide to tailing down inhaled corticosteroid therapy. Although there is some evidence that ECP levels are affected by age, smoking, circadian rhythm and seasonal variation, only smoking appears to be of clinical significance.

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Introduction

To date, the assessment of asthma has been based mainly on surrogate measures of airway inflammation such as airflow limitation or reversibility using spirometry and related techniques. Direct measurement of airway inflammation using biological markers could potentially refine asthma management and this explains current research interest in exhaled nitric oxide and eosinophil granule proteins in asthma. In recent years, clinical research has suggested an emerging clinical usefulness of eosinophil granule proteins in the assessment and management of asthma, of which eosinophil cationic protein (ECP) has been most widely characterized and researched. The aim of this paper is to systematically review the current evidence for the usefulness of ECP in asthma management and suggest future areas for clinical research.

Methods

A systematic review was performed using the Quality of Reporting of Meta-Analysis (QUORUM) Guidelines. A Medline search using the key terms eosinophil cationic protein and asthma was performed on 2 January 06, limiting the search to title or abstract of papers and to humans only. A similar search in major respiratory journals (i.e. Thorax, American Journal of Respiratory and Critical Care Medicine, European Journal of Respiratory Journal, Respiratory Medicine and Journal of Asthma) was also performed. Six hundred and eighty-eight potential papers were found. The abstracts were then reviewed based the following selection criteria: (1) ECP was used as a biological marker (regardless of body fluid sampled), (2) asthma was the index disease studied (regardless of type of asthma), (3) it was a controlled clinical study, (preferably but not limited to randomized controlled trials) and (4) ECP was assessed as a diagnostic, assessment or management tool. Only 169 articles potentially satisfied the selection criteria. Full-text versions of the papers were then obtained and reviewed. Relevant references cited in the papers but not identified by the Medline search were also obtained and reviewed. After full-text review, only 53 papers were found to fulfill the selection criteria. A flow chart detailing the systematic review process is found in Fig. 1. The results of the review are divided into two sections: the first part gives an overview of the biological properties and issues of sampling ECP from various body fluids and the second part reports the uses and limitations of ECP in asthma management.

Results

Properties, characteristics and genetics of ECP

ECP is one of the four major cationic granule proteins released by activated eosinophils (the other three being...
eosinophil protein X (EPX), eosinophil peroxidase (EPO) and major basic protein (MBP)] and is presently the most widely used clinical marker of eosinophil activity in asthma. ECP is a single-chain, zinc-containing protein with a molecular weight ranging from 16 to 22 kDa. The heterogeneity of the molecule is partially due to differences in glycosylation of three potential sites in its amino acid chain. The mechanism of action of ECP is due to its cytotoxic capacity to create pores in cell membranes. It has been shown to have cytotoxic effects against bacteria, parasites, viruses and respiratory epithelial cells. ECP also stimulates mucous production in airways and histamine release by basophils and mast cells in vitro. ECP is synthesized in eosinophil progenitors in human bone marrow and stored in specific granules in mature peripheral blood eosinophils. Triggers of eosinophil release of ECP include immunoglobulin G complexes and interleukin-5. In vitro, corticosteroids at pharmacological doses appear to be unable to inhibit ECP release from eosinophils. A more detailed account on the properties and characteristics on ECP can be found in a paper by Venge et al. The gene that codes for ECP has been located on chromosome 14q11.2 and three polymorphisms have been identified by Noguchi et al. Although, no relationship between ECP gene polymorphisms and asthma was found in this study, serum ECP levels of persons with the $\Lambda^{393}T$ allele was lower than those with the $\Lambda^{393}C$ allele. This may explain why serum ECP in some asthmatic patients is not raised and the authors suggested that ECP measurement in future asthma studies should include genotyping for $\Lambda^{393}C/T$ polymorphism.

**ECP in body fluids**

ECP can be measured in many different body fluids such as serum, plasma, sputum, saliva, nasal lavage fluid, bronchoalveolar lavage fluid and urine. The levels (normal and asthmatic) and advantages and disadvantages of ECP measurement in various body fluids are detailed in Table 1.
Serum and plasma ECP

The levels of ECP in serum is often five to ten times of those in plasma because eosinophils in blood containing EDTA are inactivated and do not continue their extracellular release of ECP ex vivo. Hence, serum ECP levels reflect circulating ECP and the propensity of active eosinophils to release ECP ex vivo, which may explain why the clinical information obtained using serum ECP have been found to be more clinically useful than plasma ECP. ECP secretion in blood samples can also increase by two to four times at 37°C relative to 22°C when stored for an hour. Therefore, serum sampling of ECP should be standardized as far as possible because levels can vary depending on the storage time and ambient temperature. The serum ECP level for both non-atopic adults and children is less than 20 μg/L.

Sputum ECP

ECP is also present in the sputum. The median range of ECP in sputum of non-asthmatics is about 200–350 μg/L, which is higher than serum levels. A major challenge with sputum measurement of ECP is that it has to be induced in non-productive subjects with nebulized saline. Immediate mucous and cell dispersion and cationic detergent addition are needed for sputum to avoid ECP destruction or variable attachment to test-tube walls and mucus. There is wide overlap between reported values of ECP in normal subjects which is likely due to the different reported methods of analysis. Standardization of sputum ECP analysis is needed before it is used more widely and important aspects of this issue are well discussed in a recent paper by a sputum analysis working group led by Kelly et al.

Salivary ECP

ECP has also been measured in saliva in adults and has been found to correlate with asthma severity. Saliva ECP ranges from 250 to 500 μg/L and levels were associated with presumed activity of disease as recorded by alteration of taken dose of inhaled corticosteroids (ICS). The reason why

### Table 1 Levels (normal and asthmatic), advantages and disadvantages of ECP measurement in various body fluids.

<table>
<thead>
<tr>
<th>Body fluid</th>
<th>Range of ECP levels (μg/L)</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Asthmatics</td>
<td></td>
</tr>
<tr>
<td>Serum¹¹,¹³,¹⁴</td>
<td>2–20</td>
<td>&gt;20</td>
<td>Reflects propensity of activated eosinophils to release granule proteins</td>
</tr>
<tr>
<td>Plasma¹¹,¹⁴</td>
<td>5–10 times lower than in serum</td>
<td></td>
<td>Reflects true levels of ECP in blood</td>
</tr>
<tr>
<td>Sputum¹⁵–¹⁷</td>
<td>20–1280</td>
<td>76.8–32,000</td>
<td>High levels of ECP found</td>
</tr>
<tr>
<td>Saliva¹⁷</td>
<td>250–450</td>
<td>450–850</td>
<td>- High levels of ECP found</td>
</tr>
<tr>
<td>Nasal lavage fluid²⁰–²³</td>
<td>0–60</td>
<td>90–120*</td>
<td>- Easy and painless collection process</td>
</tr>
<tr>
<td>Broncho-alveolar lavage fluid²⁴,²⁵</td>
<td>1–3</td>
<td>5–100</td>
<td>Moderate levels of ECP found</td>
</tr>
<tr>
<td>Urine³,¹³,²⁰</td>
<td>—</td>
<td>—</td>
<td>Not useful. Urine eosinophil protein X (EPX)/urine creatinine ratio is more useful. However, urinary EPX is not widely used in asthma research</td>
</tr>
</tbody>
</table>

*Expressed as μg of ECP per g of protein.²¹
levels of ECP in sputum and saliva are 10 times higher than serum is unclear. However, Schmekel et al. postulated that sputum and saliva ECP reflected a pathophysiological consequence of eosinophils present in the whole extent of the airways and oral mucosa. Another possible reason is that circulating eosinophils in blood retain their granule contents until they reach diseased tissue, a hypothesis supported by a recent study by Malm-Erjefalt et al. The measurement of ECP in saliva is not well established and needs standardization before it becomes an accepted biological medium of assaying ECP in asthmatics.

**ECP in nasal and broncho-alveolar fluid**

ECP can also be found in nasal and broncho-alveolar lavage fluids. However, the collection of these fluids by nasal and broncho-alveolar flush is highly invasive and unlikely to enter mainstream clinical use. Moreover, the measurement of ECP in nasal and broncho-alveolar fluid is not well standardized in terms of dilution frequency and internal standards. Nasal lavage ECP was previously found to poorly correlate with asthma severity, although a recent study showed nasal lavage ECP correlated with spirometric parameters.

**ECP in urine**

Urinary ECP has been found to be inferior to urinary EPX (also known as eosinophil-derived neurotoxin (EDN)) as a marker of eosinophil turnover and activity in asthma. Hence, ECP in urine is not useful as a marker of asthma severity.

**Clinical significance of ECP in asthma**

**ECP and airway inflammation**

Numerous studies have shown that ECP correlates well with airway inflammation in asthma. Serum ECP was found to directly correlate with activated eosinophils in bronchial mucosa biopsied from adult asthmatic patients. Serum ECP has also been found to be a more sensitive marker of asthma severity than peripheral blood eosinophil counts in acute exacerbations in children. In this study that compared serum ECP and peripheral blood eosinophil count in 46 asthmatic children, the ratio of ECP to eosinophil count was significantly greater during acute exacerbation than during clinical remission. The authors hypothesized that eosinophils released ECP preferentially during acute exacerbations than during clinical remission.

Sputum ECP has been found to be better than sputum eosinophils in characterizing oral corticosteroid dependent asthmatics with recent exacerbations. In a study that compared serum ECP and sputum ECP with asthma severity using symptom scoring, sputum eosinophils and spirometry (FEV, and FEV,VC), sputum ECP was found to be a more sensitive marker of airway inflammation and asthma severity than serum ECP.

Serum ECP rises during asthma exacerbations caused by upper respiratory infections. However, a study between adult asthmatics experiencing exacerbations caused by para-influenza virus 3 (PIV3) and non-PIV3-induced exacerbations found no significant difference in their sputum ECP levels. Another study found that serum ECP was raised in children with asthma but not in infants with respiratory syncytial virus (RSV) bronchiolitis. These studies suggest that ECP levels are raised in virus-induced airway inflammation in asthma but are not affected by the type of virus triggering asthma nor raised in bronchiolitis.

**ECP and airway hyper-responsiveness**

Although ECP correlates with airway inflammation, studies in primates have found no correlation between ECP and airway hyper-responsiveness. In a population-based study on atopy of 1189 persons in Denmark, there was no association between serum ECP and bronchial hyper-reactivity as measured with methacholine challenge tests. Similar findings have been found in other studies on asthma. The reason is probably because ECP is not significantly related to the release of bronchoactive chemical mediators that increase the responsiveness of airway smooth muscle. In contrast, MBP which is another major cationic protein of eosinophil granules, has been found to induce airway constriction and hyper-responsive-ness in primates.

**ECP as an asthma marker**

**ECP as a diagnostic tool**

There is now a good body of evidence that sputum ECP is raised in asthmatics, both in children and adults, and is increased in classic asthma, cough-variant asthma and occupational asthma. However, ECP is also raised in other atopic diseases like allergic rhinitis and in pre-school children with recurrent wheezing. ECP is also elevated in conditions that are not associated with eosinophil inflammation or atopy. For example, ECP in nasal lavage fluid is also associated with rhinovirus infections and bacterial sinusitis. Hence, ECP is not a useful diagnostic marker for asthma because of its poor specificity. It is worth noting that serum ECP is poorly associated with exercise-induced asthma, atopic eczema and food allergies. In allergic rhinitis, nasal lavage ECP has been found to increase with nasal allergen challenge and seasonal allergen exposure but reduced by immunotherapy.

**ECP as an assessment tool**

There is now a good body of evidence that ECP can be used to assess asthma severity. Serum ECP has been shown to correlate with severity of asthma as classified by STEP classification of the National, Heart, Lung and Blood Institute (NHLBI), FEV, FEV,VC, peripheral blood eosinophil count and severity of atopy based on wheal size and number of positive prick tests. Sputum ECP has also been found to correlate with asthma severity based on NHLBI and Japanese Society of Allergology criteria, PEFR, sputum eosinophils, need for oral corticosteroid therapy and FEV in treated asthmatics. Although, ECP correlates with STEP classification of asthma severity, there is little difference in sputum ECP levels between mild persistent and moderate asthmatic patients attending an asthma clinic. Hence, ECP is useful mainly in distinguishing between mild intermittent and severe asthmatics. This finding is interesting in the light of a 10-year longitudinal cohort study by de Marco et al. which found that patients with intermittent or mild persistent asthma were a different
phenotype from moderate and severe persistent asthma, with the latter group characterized by high IgE levels, persistent cough/mucus hypersecretion and early deterioration of lung function. A recent paper reported that serum ECP was positively correlated to air-trapping in asthma as measured by quantitative high-resolution computed tomography (HRCT) which in turn, correlated with FEV1/FVC.

Serum ECP levels in an asthmatic child increases significantly during asthma exacerbation, from 27.4 ± 11.5 µg/L at clinical remission to 41.7 ± 16.9 µg/L during acute exacerbation. Sputum ECP is also significantly increased in adult oral corticosteroid-dependent asthmatics who are uncontrolled and still experiencing acute exacerbations (mean = 580 µg/L) compared to controlled oral corticosteroid-dependent asthmatics (mean = 194 µg/L).

ECP as a management tool
A study by Prehn et al. using serum ECP to guide anti-inflammatory treatment of asthma in children demonstrated the usefulness of ECP in deciding when to tail down therapy. Using a clinical algorithm, they titrated the dose of anti-inflammatory drugs ICS based on serum ECP levels taken monthly in 21 children over 12 months. Patients with persistently raised ECP > 30 µg/L remained on high doses of ICS (budesonide 400 µg bd), those with ECP between 15 and 30 µg/L were given budesonide 200 µg bd and those with ECP < 15 g/L received sodium cromoglicate only. Compliance was counter-checked by weighing the metered dose inhalers each month. During the study period, there was no asthma attacks, need for emergency room treatment, hospital admission or rescue treatment with oral steroid therapy in each of the three groups of patients. This study supports strongly the usefulness of ECP in guiding asthma therapy, considering that 45% of adult asthmatics relapse after asthma exacerbation within 8 weeks. Prehn et al. also found that patients who were non-compliant to ICS medication exhibited an increase in serum ECP. ICS was more effective than sodium cromoglicate in reducing serum ECP, improving symptoms and lung function. Several other studies also support the close relationship between ECP and use of corticosteroids, suggesting that ECP can be used as a marker of compliance to oral and ICS therapy. However, it should be noted that the suppressive effect of ICS on ECP is not sensitive enough to reflect small changes in doses.

ECP correlates with other forms on anti-inflammatory agents besides ICS and mast cell stabilizers. Sputum ECP declines with leukotriene receptor antagonist (LTRA) therapy but the correlation is weaker when compared with ICS therapy. Anti-inflammatory therapy with tacrolimus in aspirin-induced asthma is associated with declines in sputum ECP as well. Immunotherapy in asthmatic children is also associated with decline in sputum ECP levels. However, ECP is not affected by H1 anti-histamines or β2-agonists when given as add-on therapy to corticosteroid-treated asthmatics. The clinical uses of ECP are summarized in Table 2.

Factors that affect ECP levels in asthmatics

Age
There is some suggestion that ECP appears to be more sensitive to changes in asthma severity in adolescents than in pre-pubertal children. However, these findings need to be confirmed by a more rigorously designed study. A study by King et al. suggested that serum ECP does not appear to be raised in elderly asthmatic patients, but his study was based on well community-living older persons and it did not measure the severity of asthma in its subjects.

Smoking
Sputum ECP in adult asthmatics who smoke is significantly higher than in non-smoking asthmatics. Moreover, inhaled budesonide treatment reduces serum ECP and improves lung function in non-smoking asthmatics but not in smoking asthmatics. This is expected as smoking is well known to cause chronic persistent inflammation of the airways. Hence, the smoking history of subjects should be considered when conducting ECP research or interpreting ECP results.

Circadian rhythm and seasonal variation
Wolthers and Heuck reported circadian rhythm variations in serum ECP in children. They found that serum ECP levels reached a trough at 8.00 a.m. but climbed as the day progressed till the late evening and early morning, reaching a peak at 6.00 a.m. However, this circadian fluctuation is negligible when one considers that the range of ECP fluctuation in normal subjects was between 3.9 ± 0.7 and 16.3 ± 3.5 µg/L (mean ± SEM), and serum ECP in asthmatics is often raised above 20 µg/L. Serum ECP in non-atopic children aged 11–16 yr also exhibited seasonal variations with serum ECP decreasing from a median value of 14 µg/L in May to 7 µg/L in November. These seasonal variations are probably due to seasonal environmental influences rather than daylight exposure, and the levels are so low and the range so narrow that it would be insignificant when serum ECP levels are used as an inflammatory marker of asthma.

ECP and other inflammatory markers of asthma
There are many inflammatory markers of asthma currently being investigated such as nitric oxide and their metabolites, arachidonic acid metabolites such as leukotriene E4 and 8-isoprostane, chemokines such as interleukins, γ-interferon, Th2-specific macrophage-derived chemokine and eosinophil chemoattractant, eotaxin. These markers are being measured with established methods such as exhaled breath, sputum and urine, as well as novel methods such as exhaled nasal air and exhaled breath condensate. There have been no published reports on head-to-head comparisons of these markers with ECP in asthma assessment or management, mainly because many of them are still in the early stages of research, with the exception of exhaled nitric oxide (eNO). Yet, the measurement of eNO remains technically challenging, especially in children younger than 4 years, because it requires patient cooperation, strict standardized sampling techniques and a sustained minimal exhalation flow rate. On the other hand, saliva collection requires less patient cooperation and is independent of exhalation flow rate. Thus, further studies on the clinical utility of salivary ECP and its correlation with serum and sputum ECP are worthy endeavors. It is beyond the scope of this article to discuss other markers of airway
inflammation measurement but we would like to direct the reader to relevant review articles for more details.72,73

Conclusions

ECP is found in many body fluids but serum and sputum sampling are the most established to date. It reflects airway inflammation and not hyper-responsiveness, and correlates with asthma severity as measured by symptom score and spirometry. As ECP is not specific to asthma, it is not useful as a diagnostic tool. However, it has shown to be useful in assessment of compliance to most forms of anti-inflammatory therapies in asthma and in guiding the tapering of ICS in stabilized asthmatics. The response of ECP levels, rather than the absolute value, to corticosteroid therapy in asthma is probably more useful in guiding management and predicting relapse after acute exacerbations.

The use ECP in asthma has its limitations. It has been argued that eosinophils are not the only cells involved in asthma, although it is recognized that they are key players in this inflammatory airway disease.74 Not all forms of asthma have an atopic basis so the usefulness of ECP will be limited to allergic forms of asthma. Further research is needed before ECP can become a useful marker of airway inflammation to guide asthma therapy. For example, little is known about the relationship between eosinophils and ECP: Are the raised levels of ECP in asthmatics due to increased numbers of eosinophils, increased amounts of stored ECP within eosinophil granules or increased propensity of eosinophils to release ECP? We need to determine if ECP levels differ significantly between children, adults and elderly, and improve and standardize our collection methods to compare research results meaningfully. We also need to think beyond using ECP values as a single snapshot tool and begin using serial measurements to look at trends and see if they can predict asthma relapse and verify compliance and response to anti-inflammatory therapy. ECP may become even more useful if combined with other markers of asthma. ECP has the potential to meet our need for an airway inflammatory biomarker in asthma management but the key to its success lies in using what we currently know and translating it from bench to bedside.

<table>
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<td>• ECP is more sensitive than blood and sputum blood eosinophils in assessing asthma severity27,28</td>
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<td>• Sputum ECP is more sensitive than serum ECP in assessing asthma severity15</td>
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<td><strong>Airway hyper-responsiveness</strong></td>
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<td>• ECP does not correlate with airway hyper-responsiveness15,33-37</td>
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<td><strong>Diagnosis of asthma</strong></td>
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<td>• ECP is raised in other atopic diseases (e.g. allergic rhinitis, recurrent wheezing)41-43 and infections (e.g. rhinovirus infections22,44 and bacterial sinusitis27)</td>
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<tr>
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