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Eosinophil Cationic Protein Can Monitoring Efficacy of Specific Immunoterapy in Eosinophilic Esophagitis

Armentia-Medina A[1], San Miguel-Rodríguez A[2*], Martín-Armentia B[2], Martín-Armentia S[3], Angel San Miguel Hernandez[4], Iglesias-Peinado I[5] and Lozano-Estevan C[6]

[1]Allergy Unit, Hospital Universitario Río Hortega, Valladolid, Spain

[2]Research Service. Hospital Universitario Río Hortega, Valladolid, Spain

[3]Primare care. Paedriatics. Delicias. Valladolid, Spain

[4]Clinical Analysis Service. Hospital Universitario Río Hortega, Spain

[5]Pharmacy Faculty. Complutense University, Madrid, Spain

[6]Parmacy Faculty. Alfonso X el Sabio University, Madrid, Spain

***Corresponding author:**

Angel San Miguel Hernández,
Hospital Universitario Rio Hortega, Dulzaina, 47012 Valladolid, Spain,
E-mail: asanmi@saludcastillayleon.es

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1. Abstract

1.1. Background: Eosinophil Cationic Protein (ECP) has proven a useful monitor for many active eosinophilic inflammatory diseases. In the last years we have successfully treated patients suffered from eosinophilic esophagitis whit Allergen Specific Immunotherapy (AIT) and diet guided by Component-Resolved Diagnosis (CRD), that could detect possible allergens involved in eosinophilic esophagitis (EoE).

1.2. Objective: We carried out measurements of ECP after 3 years of AIT in patients with EoE and two year of suspension of this treatment without relapse of disease, in order to evaluate ECP as objective marker of improvement of the esophagitis.

1.3. Methods: One hundred and twenty-nine patients with EoE were tested for environmental and food allergens. CRD, histological and botanical analysis were performed. Clinical scores and endoscopic biopsies were performed every six months for five years. Fifteen healthy patients and 34 asthmatics due to pollen were included as control groups.

CRD-directed allergen immunotherapy (AIT) was administered in 91 EoE patients and conventional treatment (proton pump inhibitors, empiric diet, corticosteroids) in the rest of patients (n=38). Sera of all patients were collected before de therapy and after the suppression of the treatment. Randomized blind analysis of ECP was performed in all samples of treated (AIT/conventional) and controls subjects and correlated with clinical and endoscopic findings.

1.4. Results: Higher ECP levels were measured in patients with EoE (mean 46.5 ng / mL) with respect to pollen asthma (mean 19.17 ng / mL) and higher in both processes than in healthy patients ($p < 0.0001$). In patients treated with AIT, a marked decrease in CPE was observed, significantly higher than in patients with conventional therapy ($p < 0.001$). This improvement was significantly correlated with clinical and endoscopic findings of favorable evolution of the disease ($p < 0.001$)

1.5. Conclusion: Measurement of ECP can be useful in monitoring efficacy of specific immunotherapy in EoE as can be used also as a marker of activity of the disease.

2. Introduction

Eosinophils appear in large number at inflammation sites in response to certain parasitic infections. These leukocytes, when mature, reside mostly in tissues, but about 1% of the eosinophil population circulates in the blood. Activated eosinophils degranulate to release highly basic proteins into the surrounding tissue. The granular pro-

teins, which can kill parasites and some mammalian cells, might cause the tissue damage associated with asthma and other inflammatory diseases [1, 2]. Eosinophil activation accompanies a wide range of inflammatory diseases, including bronchial asthma and eosinophilic esophagitis [3, 6].

Among the four basic granule proteins, Eosinophil Cationic Protein (ECP) has proven a useful monitor for many active inflammatory diseases [7]. ECP concentrations in plasma and certain other body fluids increase during inflammatory reactions marked by activated eosinophils. Produced by eosinophils exclusively, ECP is toxic to neurons, some epithelial cell lines, and isolated myocardial cells. The positively charged protein binds to heparin and inhibits blood coagulation.

Some authors, reported significant correlations between ECP levels and bronchial hyperreactivity in mildly asthmatic patients (3).

Other authors, showed that serum ECP concentrations exceed normal, control levels in both IgE mediated and non-IgE-mediated atopic conditions. Serum ECP measurements avoid inconsistencies inherent in subjective asthma assessments [5]. Further, serum ECP concentrations can indicate the severity of certain skin disorders [8].

Eosinophilic esophagitis (EoE) is characterized by esophageal dysfunction and, histologically, by eosinophilic inflammation. Conventional treatment (proton pump inhibitors or PPIs, corticosteroids, empiric diet, dilations) did not resolved the symptoms in all patients. Environmental allergies to substances such as dust mites, animals, pollen and molds can play a role in EoE. Allergy testing for these common environmental allergies is often part of the EoE evaluation [4]. For some patients, it may seem like their EoE is worse during pollen seasons [6, 9-11].

Six years ago129 patients with EoE were tested for environmental and food allergens [9]. Component Resolved Diagnosis (CRD), histological and botanical analysis was performed. Microscopic examination of esophageal biopsies of 129 adult's patients with EoE, 82 of them with seasonal exacerbation, and 100 controls, with gastroesophageal reflux without eosinophilic infiltrate, were made to verify the presence of callose (polysaccharide abundant in pollen tubes but absent in animal tissues) in the esophagus.

CRD detected pollen allergens in 87.6% of patients with EoE and lipid transfer proteins (LTPs) of common Mediterranean foods such as peach, hazelnuts, walnuts and wheat in 19.4%. Callose from pollen tubes was found in 65.6 % of biopsies. Alteration of the mucosal barrier in EoE might cause the penetration of pollen grains, spores and other food particulars into the esophageal tissues. Eosinophils seemed to act as if they were responding to parasitic infections [9]. In EoE patients, anatomopathological studies searching for intrusion to plant foods and pollen, and specific-guided diet and immunotherapy after plant structures detection in biopsies were useful to identify the possible cause of EoE [3].

We began CRD guided specific immunotherapy in these patients. After CRD-guided elimination diet and/or AIT, 101 (78.3%) EoE patients showed significant clinical improvement ($p< 0.017$) and 97 (75.2%) were discharged (negative biopsy, no symptoms, no medication) without relapse. AIT-treated patients had better outcomes (odds ratio 177.3, 95% CI 16.2–1939.0) that the patients with conventional treatment.

We have keep refrigerated the sera of the patients before the treatment, in the moment of the first diagnosis, and now we compared the levels of ECP in 36 patient's sera after 3 years of immunotherapy and 2 years of suppression of AIT due to resolution of this disease (negative clinical and biopsy findings) with 38 samples of patients that were treated with conventional therapy in the same period, that continue with symptoms.

The aim was to obtain an objective marker of improvement of the EoE that may obviate or reduce the need of endoscopic biopsies.

3. Material and Methods

One hundred and twenty-nine patients with EoE were tested for environmental and food allergens. CRD, histological and botanical analysis were performed. Clinical scores and endoscopic biopsies were performed every six months for five years. Fifteen healthy patients and 34 asthmatics due to pollen were included as control groups. These patients were provided with sufficient information to decide whether to receive usual therapy (oral corticosteroids, proton-pump inhibitors, 6-food diet) or CRD-guided diet and AIT. The patient information sheet and informed consent form, and a further informed consent for histological biopsy study, were approved by the University Hospital Rio Hortega Ethics Committee.

CRD-Directed Allergen Immunotherapy (AIT) was administered in 91 EoE patients and conventional treatment (Proton pump inhibitors, empiric diet, corticosteroids) in the rest of patients (38). Sera of all patients were collected before de therapy and after the suppression of the treatment.

We compared now the levels of ECP in 36 patient's sera after 3 years of immunotherapy and 2 years of suppression of AIT due to resolution of this disease (negative clinical and biopsy findings) with the levels of ECP in the 38 patients that followed conventional treatment.

Randomized blind analysis of ECP was performed in all samples of treated (AIT/conventional) and controls subjects and correlated with clinical and endoscopic findings.

The serum samples were collected, after blood extraction and subsequent centrifugation, in polystyrene tubes for storage, instead of glass tubes, to avoid the decrease of ECP values. The samples were frozen in a library at -20ºC, until the end of the study [12].

The method to measure to ECP in serum was Immulite 2000 ECP Siemens, German, a solid-phase, two-site chemiluminiscent immunometric assay, who were carried out following the manufacturer's recommendations. The expected values in our laboratory in healthy individuals was 8.33+/-5.86 ng/mL We also followed the National

Committee for Clinical Laboratory Standards Procedures for the collection of diagnostic blood specimens [13].

The measurement technique of ECP with Immulite 2000, presents a calibration range up to 200 ng/mL and analytical sensitivity 0.2 ng/mL.

4. Statistical Analysis

ECP levels were described as mean ± standard deviation (SD). At baseline, ANOVA with Bonferroni post-hoc test was performed to detect differences between groups. The non-parametric Wilcoxon test for paired samples was used to compare pre and post AIT means. To determine de best cut-off values to detect EoE, a Receiving Operating Characteristic (ROC) curve analysis was performed, and sensibility, specificity and predictive values were calculating. For all tests, a significance level was established in $p<0.05$.

5. Results

The imprecision study of the ECP inter and intraserie technique was performed for two control levels, 10 times of each. The results are shown in (Table 1).

Before treatment, mean ECP levels were higher in patients with EoE (46.6 ± 37.7 ng/ml) in relation to asthmatic groups (19.2 ± 18.9 ng/ml) and healthy controls (8.3 ± 5.9 ng/ml), with a significant intergroup difference ($p <0.0001$).

In addition, the ECP levels were higher in patients with EoE than both in asthmatics due to pollen ($p <0.001$) and in healthy controls ($p<0.001$), while no differences were detect between asthmatic and healthy subjects (Figure 1).

It is obvious that the ideal is to work with diagnostic tests of high sensitivity and specificity, but this is not always possible. In general, screening tests must be highly sensitive in order to capture all patients. A very sensitive test will be especially appropriate in those cases in which not diagnosing the disease can be fatal for patients, or in diseases in which a false positive does not produce serious disorders.

On the other hand, specificity refers, as previously noted, to the probability that a healthy subject will be appropriately classified. In general, the confirmatory tests of the diagnosis must be of high specificity, to avoid false positives. High specificity tests are necessary in serious diseases but without available treatment that makes them curable, when there is great interest in knowing the absence of disease or when diagnosing a patient from a disease that does not really suffer can have serious consequences.

To detect EoE at baseline, ECP values showed an Area Under the Curve of 0.824. (Figure 2) shows the ROC curve of the ECP.

The important thing is to work with diagnostic tests of high sensitivity and specificity, but this is not always possible. In general, screening tests must be highly sensitive in order to capture all patients. A very sensitive test will be especially appropriate in those cases in which not diagnosing the disease can be fatal for the patients, as it happens with dangerous but treatable diseases in which a false positive does not produce serious disorders for the patient. On the other hand, specificity refers, as previously noted, to the probability that a healthy subject will be appropriately classified. In general, the confirmatory tests of the diagnosis must be of high specificity, to avoid false positives. High specificity tests are necessary in serious diseases but without available treatment that makes them curable, when there is great interest in knowing the absence of disease or when diagnosing a patient who does not really suffer it can have consequences.

Therefore, when choosing the cut off, we decided to put it at 20 ng / ml or 13 ng / ml (Table 2). If the cut off is established in 13 ng/ml, with a sensitivity of 90.7% and a specificity of 67.3%. The best cut off was established in 20 ng/ml, with a sensitivity of 75.9% and a specificity of 77.6% (Table 1).

Favourable progression of the disease was more frequent in patients with AIT (39/40 patients, 97.5%) than in other therapies (15/27 patients, 55.6%) ($p<0.001$). Patients treated with AIT showed a significant decrease in ECP levels ($p <0.001$), while patients with conventional treatment showed a significant but slight increase after treatment (Table 3).

By the other hand, this significant decrease in ECP levels was equally detected in patients with either favourable or unfavourable disease progression ($p=0.008$ and $p <0.001$, respectively) (Table 3). Besides, an absence of relation between baseline ECP and the progression of disease was detected by ROC curve analysis (AUC, 0.441; CI95%, 0.314 – 0.569; $p=0.372$).

Table 1: Study of imprecision of the ECP technique.

Controls	Interserie (n=10)	Intraserie (n=10)
High	496.2 (CV=7.6%)	499.3 (CV=9.3%)
Low	6.91 (CV=2.74%)	7.02 (CV=4.1%)

Table 2: Diagnostic Test Study of ECP to detect EoE

	ECP = 20 ng/mL		ECP = 13 ng/mL	
	Value (%)	CI95%	Value (%)	CI95%
Sensitivity	75.9	63.6 – 88.2	90.7	82.1 – 99.4
Specificity	77.6	64.8 – 90.	67.3	53.2 – 81.5
Positive predictive value	78.8	66.8 – 90.9	75.4	64.1 – 86.6
Negative predictive value	74.5	61.6 – 87.4	86.8	74.8 – 98.9
Positive likelihood ratio	3.38	1.97 – 5.81	2.78	1.84 – 4.19
Negative likelihood ratio	0.31	0.19 – 0.51	0.14	0.06 – 0.32

Table 3: Statistics of related samples, in patients with Pre and Post treatment ECP levels

	N	**Pre treatment**	**Post treatment**	**Sig.**
All patients	45	41.6 ± 32.4	29.4 ± 30.3	<0,001
Patients with AIT	26	37.7 ± 29.5	14.3 ± 11.9	<0.001
Patients without AIT	19	46.8 ± 36.1	50.1 ± 35.6	<0.001
Patients with unfavourable progression	9	36.9 ± 15.5	26.2 ± 13.4	0.008
Patients with favourable progression	36	42 ± 35.5	30.2 ± 33.3	<0.001

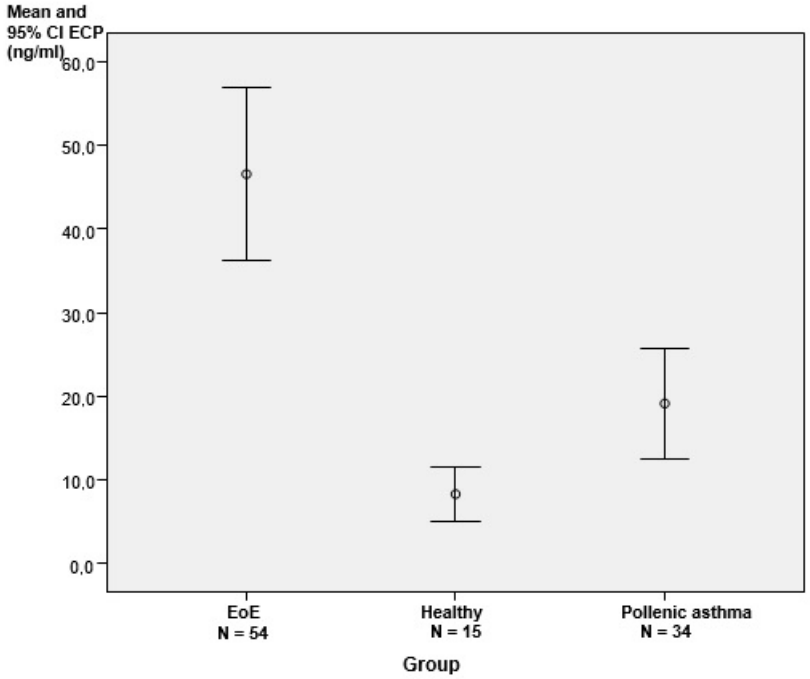

Figure 1: Mean and 95%CI levels of ECP (ng/mL) in healthy subjects, asthmatics by pollen and EoE patients.

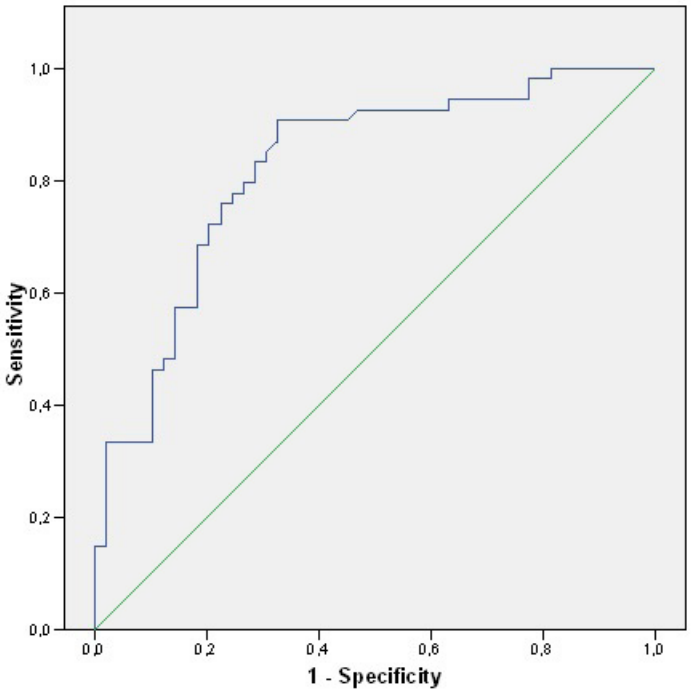

Figure 2: Curve ROC analysis to detect EoE by determining ECP (ng/mL).

6. Discussion

Eosinophilic esophagitis is a recognized allergic disease. Currently the only way to diagnose EoE is with an endoscopy and biopsy of the esophagus.

Eliminating major food allergens from the diet before any food allergy testing is an accepted treatment of EoE. The foods excluded usually include dairy, egg, wheat, soy, peanut, tree nuts and fish/shellfish. These diets have been shown to be helpful in some treating EoE, although they can be very difficult to follow. Foods are typically added back one at a time with follow up endoscopies to make sure that EoE remains in control but relapse is very frequent.

No medications are currently approved by the U.S. Food and Drug Administration (FDA) to treat EoE. However, medications have been shown to reduce the number of eosinophils in the esophagus and improve symptoms. Corticosteroids, which control inflammation, are the most helpful medications for treating EoE. Swallowing small doses of corticosteroids is the most common treatment. Different forms of swallowed corticosteroids are available. At first, higher doses may be needed to control the inflammation but the higher doses are linked with a greater risk of side effects.

Proton Pump Inhibitors (PPIs), which control the amount of acid produced, have also been used to help diagnose and treat EoE. Some patients respond well to PPIs and have a large decrease in the number of eosinophils and inflammation when a follow up endoscopy and biopsy is done. However, PPIs can also improve EoE symptoms without making the inflammation any better [4, 9].

Research in recent years has contributed to a better understanding of EoE assessment of disease activity, but it is necessary to advance in evaluation of minimally invasive diagnostic tools, and new therapeutic approaches.

We have found that CRD-directed allergen immunotherapy (AIT) and/or elimination diet was efficient in treating EoE patients and was well tolerated. Biopsies analysis using plant histology methods may show pollen tubes in the esophageal mucosa. Eosinophils seemed to act as if they were responding to parasitic infections. Component-resolved diagnosis (CRD) with microarrays could detect possible allergens involved and indicate an elimination diet and allergen immunotherapy (AIT).

In all way of treatments should be necessary monitoring their efficacy. The most common methods are based in clinical subjective scores and endoscopy with biopsy. We proposed a simple analytical method, measurement of ECP.

Among the four basic granule proteins, Eosinophil Cationic Protein (ECP) has proven a useful monitor for many active inflammatory diseases [14] and it is produced by eosinophils exclusively. Several studies report high individual and group correlations between ECP levels and clinical asthma symptoms, such as increases in Peak Expiratory Flow (PEF), inhaled β2-agonist, airway responsiveness, and spirometry [2, 5, 14]. Atopic serum samples have higher ECP levels than nonatopic control samples, even when the circulating eosinophil count remains within the normal range [5]. In seasonal asthmatic patients, ECP measurements reflect changes in disease activity throughout the year. Significant correlations between ECP levels and bronchial hyperreactivity in mildly asthmatic patients have been reported [3].

Serum ECP measurements avoid inconsistencies inherent in subjective assessments and can indicate the severity of certain skin disorders [15-17].

The objective nature of serum ECP measurements presents an advantage over the subjective clinical measures, which are prone to inconsistencies arising from the broad range in individual investigator and patient assessments.

Several unmet needs are to be solved urgently in EoE, as finding a non-invasive disease-monitoring methods and biomarkers for routine practice, the development or new therapies, novel food allergy testing to detect triggering foods, drug, and doses required for initial therapy and safety issues with long-term maintenance therapy, amongst others. Besides, multidisciplinary management units of EoE, involving gastroenterologists, pediatricians, allergists, pathologists, dietitians, and specialists are needed.

7. Conclusions

Measurement of ECP can be useful in monitoring efficacy of specific immunotherapy in EoE as can be used also as a marker of activity of the disease.

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