

No association between serum eosinophil cationic protein and atopic dermatitis or allergic rhinitis in an unselected population of children

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ABSTRACT

Background In order to obtain background references when dealing with serum eosinophil cationic protein (s-ECP) measurements in children with allergic diseases, population-based studies are important. The objectives of our study were to explore the strength of associations between the s-ECP level and atopic dermatitis (AD), allergic rhinitis (AR) and asthma in an unselected northern Norwegian schoolchildren population.

Methods s-ECP was sampled from 396 schoolchildren aged 7–12 years from Sør-Varanger community, northern Norway as a part of a population-based study of allergy. In advance, anamnestic information concerning a history of AD, AR and asthma were obtained. The children underwent a clinical investigation, including skin prick tests and peak expiratory flow measurements, where the presence of AD, AR and asthma were evaluated. The associations of these diseases to the s-ECP values were examined in bivariate statistical analysis.

Results No statistical significant associations were detected in bivariate analysis between s-ECP and AD, AR or asthma: the mean s-ECP in children without self-reported AD/AR/asthma was 4.6 µg/L [95% confidence interval (CI) 4.0–5.2]. The mean s-ECP in children with self-reported AD or AR or asthma was 5.2 µg/L (95% CI 4.1–6.2), 4.6 µg/L (95% CI 3.5–5.7) and 6.4 µg/L (95% CI 4.4–8.3), respectively. The highest mean s-ECP level was measured in children with clinically diagnosed asthma; 7.1 µg/L (95% CI 4.0–10.3). Above the 75-percentile level of s-ECP, only 17.2% of the children had a history of asthma.

Conclusions In this unselected children population, the occurrence of AD or AR was not reflected by an increase in the s-ECP level. The s-ECP was increased in children with asthma, but was not statistically significant. Furthermore, the majority of children with high s-ECP values were not asthmatics. We conclude that the associations between s-ECP and allergic diseases are weak in an unselected population of children.

Key words: allergic rhinitis, asthma, atopic dermatitis, eosinophil cationic protein, Norway, population-based, schoolchildren

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Introduction

Atopic diseases are chronic or chronically relapsing skin and respiratory disorders, and eosinophilic inflammation is one of the hallmarks of these diseases. When activated, these cells release several mediators and among them is eosinophil cationic protein (ECP). The correlations of this mediator to atopic dermatitis (AD), allergic rhinoconjunctivitis (AR) and asthma are widely studied. Serum ECP (s-ECP) is shown to be increased

in children with asthma, AR and AD.^{1–3} However, in the vast majority of studies, s-ECP is measured in hospitalized children, and studies of s-ECP and asthma and allergy in unselected populations of children are few.

We present a population-based study of s-ECP and atopic diseases in schoolchildren from a community in north Norway. The aim of the study was to investigate the strength of association between mean s-ECP level and AD, AR and asthma in an unselected population of children.

Methods

From February 1992 to March 1993, a clinical follow-up study was conducted in Sør-Varanger community, northern Norway. The community comprised 575 primary school children aged 7–12 years. All children had a year in advance been enrolled in a questionnaire-based study of asthma and allergy ($n = 551$).⁴ In the study presented in this paper ($n = 424$), a clinical examination of this schoolchildren population was performed, including blood sampling and skin prick test (SPT). In advance, the physician filled in a one-paged questionnaire concerning a history of asthma, AR and AD, age of onset and symptoms during the last year, and information about indoor environment and family history of allergic disease was obtained.^{5,6} All information given in the questionnaires was discussed and reviewed with each child and their escort (by a dermatologist: LKD). An affirmative answer to the questions 'Have you had eczema/asthma/hay fever?' will be referred to as self-reported disease.

The diagnosis of asthma, AR and AD was made upon the results of the clinical examination and SPT. The clinical examination of the children was taken care of by an experienced dermatologist (LKD). Asthma was diagnosed if the child had had three or more recurring attacks of bronchial obstruction causing wheezing, coughing, or heavy breathing due to external factors such as animal dander, pollen, house dust or food. A pathological peak expiratory flow (PEF) was used as a parameter for a diagnosis of asthma in children reporting earlier bronchial obstruction or suspected symptoms during the clinical examination. AR is here defined as episodic rhinorrhoea associated with nasal stuffiness and sneezing in response to known or strongly suspected allergen(s) and often accompanied by lacrimation and red and itchy eyes.⁶ The diagnosis of AD was made according to Hanifin and Rajka.⁷ The disease severity was assessed using the score system given by Rajka and Langeland, i.e. percentage area of the body affected, severity of pruritus and the course of the eruption.⁸ The course of the eruption was categorized into three levels: (i) more than 3 months of remission over a period of 1 year (mild); (ii) less than 3 months of remission over a period of 1 year (moderate); and (iii) continuous eruption (severe).

The SPT were undertaken with following allergens (Allergologisk Laboratorium A/S, Hørsholm, Denmark): house dust mite (*Dermatophagoides pteronyssinus*), animal dander (cat, dog), mugwort pollen, birch pollen, grass (Timothy) pollen, mould (*Cladosporium herbarum*), and the following food allergens: fish (cod), hen's egg, cow's milk and white flour. As a positive reference, a histamine dihydrochloride solution was used.⁶ The negative control was an allergen diluent with 0.9% NaCl and 0.5% phenol. The skin was punched through a droplet of extract with a Prick Lancette 1 mm needle (Ewo care AB) on the volar side of the forearm and the reactions were recorded after 15 min. The histamine-induced weal was recorded as half the sum of the largest, plus the perpendicular diameter. The weal reactions elicited by the allergen preparation were recorded as

3+ when the weals were of the same order of size as that of the histamine reference. A 2+ reaction was half of, and a 4+ twice the histamine reaction. A reaction was considered positive if it was 2+ or greater.⁹

The blood samples for s-ECP and s-IgE measurements were collected as follows. Through a venous puncture, blood was sampled in Vacutainer® tubes and inverted five times before stored at room temperature for 30 min.^{10,11} Afterwards, the tubes were centrifuged to separate the serum, which was stored at -20°C until analysed. The values of s-ECP and s-IgE were determined using the Pharmacia CAP-system, FEIA (Pharmacia AB, Uppsala, Sweden). Concerning s-ECP, the limit of detection was $1.0\text{ }\mu\text{g/L}$, which thus was the lowest level recorded. According to the manufacturer's recommendations, an upper limit of $180\text{ }\mu\text{g/L}$ for normal s-IgE was chosen.

Because the s-ECP distribution was skewed left and hence not normally distributed (fig. 1), all statistics were by non-parametric bivariate analysis of variance, i.e. Wilcoxon scores, and handled by the SAS software package.¹² The study was approved by the Ethical Committee of Tromsø University.

Results

The s-ECP level was measured in 396 children (47% girls) and the mean level was $4.9\text{ }\mu\text{g/L}$ (SD 5.3, 95% confidence interval 4.4–5.5). The s-ECP level was not associated neither to gender nor to age. Moreover, we did not find any statistical significant association between neither s-ECP level and SPT, nor s-ECP and s-IgE (data not shown).

There was no statistically significant association between s-ECP and asthma. However, the mean s-ECP level was elevated in asthmatics compared with non-asthmatics (Table 1) with the highest mean level detected in children with diagnosed asthma at the clinical consultation. Among the asthmatics, the mean

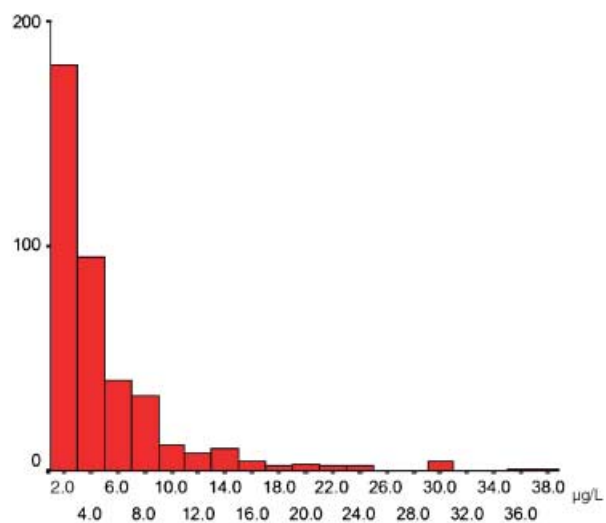


fig. 1 Histogram of s-ECP distribution.

Table 1 Mean serum eosinophil cationic protein with 95% confidence interval in children with asthma, allergic rhinoconjunctivitis (AR) and atopic dermatitis (AD)

	<i>n</i>	Mean (µg/L)	95% confidence interval
Self-reported asthma	49	6.4	4.4–8.3
Clinically diagnosed asthma	27	7.1	4.0–10.3
Self-reported AR	71	4.6	3.5–5.7
Clinically diagnosed AR	20	3.8	2.0–5.7
Self-reported AD	142	5.2	4.1–6.2
Clinically diagnosed AD	96	4.5	3.5–5.6
Children without self-reported asthma/AR/AD	201	4.6	4.0–5.2

s-ECP level did not differ between children with a positive and negative SPT. Above the 75-percentile (s-ECP 6.0 µg/L), only 17.2% of the children had a history of asthma.

The mean s-ECP level was neither elevated in children with self-reported AD/AR nor in children with AD/AR diagnosed at the clinical examination (Tables 2 and 3). Concerning children with AD, no associations were detected; neither between s-ECP and the severity score, nor between s-ECP and the three level course of the eruption categorization.

Discussion

In this unselected population-based study of 396 children aged 7–12 years, the occurrence of AD or AR was not reflected by an

increased s-ECP level. The level of s-ECP was increased in children with asthma, but was not statistically significant. Furthermore, the majority of children with high s-ECP levels were not asthmatics.

The relationship between s-ECP measurements and the plasma coagulation process was investigated by Swedish researchers.¹¹ They concluded that the raised ECP levels in serum are unrelated to the coagulation process, but are due to the continuous secretion *ex vivo* of ECP from active eosinophils. The s-ECP values in this study were measured after 30 min coagulation time at room temperature before spinning. This protocol was according to the recommended standards at the time of the study.¹⁰ The mean s-ECP level in our study is lower than in other studies where 60 min coagulation time has been used.¹³ Anyway, this is compensated for by a large study population (*n* = 396 children), and we will argue that our study is descriptive of the associations that exist between s-ECP and mild AD, AR and asthma in children.

The theory that asthma is associated with an elevation of the s-ECP level is widely accepted. A population-based study of schoolchildren in Norway found significantly higher mean s-ECP level in asthmatic than non-asthmatic children.¹³ However, the study did not find any difference in the s-ECP level in atopic and non-atopic asthmatic children. A study from Finland showed a corresponding decrease of the s-ECP level in patients treated with inhaled corticosteroids, indicating that decreased bronchial inflammation reflects changes in the s-ECP level.¹⁴ The design of this clinical study focused on AD.

Table 2 Mean serum eosinophil cationic protein values in children with atopic dermatitis (AD) and non-AD, with SD and *P*-value

	<i>n</i>	Mean (µg/L)	SD	<i>P</i> -value
Children with a history of AD	142	5.2	6.3	NS
Children without a history of AD	254	4.8	4.7	
Children with a history of AD and without a history of asthma/AR	90	5.7	7.2	NS
Children with no history of asthma/AR/AD	201	4.6	4.2	
Children with AD at the time of investigation	96	4.5	5.1	NS
Children without AD at the time of investigation	300	5.1	5.4	
Children with AD at the time of investigation and without a history of asthma/AR	59	4.7	5.8	NS
Children with no history of asthma/AR/AD	201	4.6	4.2	

NS, not significant; AR, allergic rhinoconjunctivitis.

Table 3 Mean serum eosinophil cationic protein values in children with allergic rhinoconjunctivitis (AR) and non-AR, with SD and *P*-value

	<i>n</i>	Mean (µg/L)	SD	<i>P</i> -value
Children with a history of AR	71	4.6	4.6	NS
Children without a history of AR	322	5.0	5.5	
Children with a history of AR and without a history of asthma/AD	28	4.0	2.9	NS
Children with no history of asthma/AR/AD	201	4.6	4.2	
Children with AR at the time of investigation	20	3.8	3.9	NS
Children without AR at the time of investigation	376	5.0	5.4	
Children with AR at the time of investigation and without a history of asthma/AD	7	2.2	0.8	NS
Children with no history of asthma/AR/AD	203	4.6	4.2	

AD, atopic dermatitis.

Therefore, two important issues concerning asthma were left out: (i) a detailed examination of current use of inhaled corticosteroids was not recorded, and (ii) instead of spirometry and measurements of bronchial responsiveness, peak expiratory flow measurements were performed. The majority of asthmatic children in our study were treated with antiasthmatic drugs, including inhaled corticosteroids, but unfortunately, this could not be adjusted for in the statistical analysis as this confounder was not recorded. Because of these uncertainties concerning the asthma label and treatment, our results concerning associations between s-ECP and asthma must be handled with care.

Some studies have shown that AR and AD can lead to an increase in the s-ECP level.^{1,3,15} In contrast, a German study comparing the Severity Scoring of Atopic Dermatitis and s-ECP level, did not find any correlation.¹⁶ Nevertheless, a recent study from Japan reported significant elevation of the s-ECP level in patients with severe and moderate, but not mild AD.¹⁷ In our study most children had mild symptoms with flexural dermatitis. That is, two-thirds of the children had mild AD, one-third moderate, and only three children had severe symptoms of AD.⁵ The lack of association between AD and s-ECP may partly be explained by this fact.

Despite asthma and allergy, several other conditions such as parasitosis and mother's addiction to tobacco, are associated with increased s-ECP.¹⁸ In our study population, only 17.2% of the children with s-ECP level above the 75-percentile, had a history of asthma. In order to investigate further the relevance of s-ECP measurements in children, population-based studies which include all known parameters, i.e. asthma and allergy, parasitosis, etc., is recommended. The aim of controlling all determinants of s-ECP is required to explore further the place of s-ECP measurements in childhood asthma and allergy.

It is important to remember that this study was done in an unselected children population. Most studies on s-ECP in children are undertaken in a hospital setting where it is likely to assume that the children tested have more severe asthma or allergic disease. However, in order to explore the rationale of s-ECP testing in community medicine, trials on unselected populations are essential. Our study indicates that s-ECP measurements have limited value as a screening procedure in asthma and allergy in children. Furthermore, as the associations between s-ECP and asthma are at best weak in cases of mild disease and non-existent in mild AD and AR, s-ECP measurements may be of little interest in most clinical settings concerning asthma and allergy in children in primary care.

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