

Simultaneously elevated exhaled nitric oxide and serum-eosinophil cationic protein relate to recent asthma events in asthmatics in a cross-sectional population-based study

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Summary

Background We have reported that increased fraction of exhaled nitric oxide (FeNO), a measure of TH₂-driven airway inflammation, and blood eosinophil count, a marker of systemic eosinophil inflammation, correlated with asthma attacks in a population-based study.

Objective To investigate the relation between simultaneously elevated FeNO and serum eosinophil cationic protein (S-ECP) levels and asthma events among asthmatics.

Methods Measurements of FeNO (elevated ≥ 25 ppb) and S-ECP (elevated ≥ 20 ng/mL) were performed in 339 adult asthmatics. Asthma events (attacks and symptoms) were self-reported.

Results Simultaneously normal S-ECP and FeNO levels were found in 48% of the subjects. Subjects with simultaneously elevated S-ECP and FeNO (13% of the population) had a higher prevalence of asthma attacks in the preceding 3 months than subjects with normal S-ECP and FeNO (51% vs. 25%, $P = 0.001$). This was not found for subjects with singly elevated S-ECP ($P = 0.14$) or FeNO ($P = 0.34$) levels. Elevated S-ECP and FeNO levels were independently associated with asthma attacks in the preceding 3 months after adjusting for potential confounders (OR (95% CI) 4.2 (2.0–8.8)).

Conclusions Simultaneously elevated FeNO and S-ECP levels were related to a higher likelihood of asthma attacks in the preceding 3 months. This indicates that there is a value in measuring both FeNO and systemic eosinophilic inflammation in patients with asthma to identify individuals at high risk of exacerbations.

Clinical relevance FeNO and S-ECP are markers for inflammation in asthma, but are dependent on different inflammatory pathways and weakly correlated. Simultaneous measurements of both offer better risk characterization of adult asthmatics.

Keywords asthma, clinical immunology, epidemiology

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Introduction

Airway inflammation, obstruction and bronchial hyper-responsiveness are hallmarks of asthma. The goal of asthma treatment is to achieve asthma control [1], and the main treatment is anti-inflammatory, for example inhaled corticosteroids. However, not all patients achieve asthma control at the same time as overtreatment increases the risk for side effects. Therefore, tools

for monitoring inflammatory activity are needed for efficient treatment, and to avoid unnecessary dose-related side effects.

Asthma is a heterogeneous disease that covers several different pathological processes with reversible restricted airflow in the airways and with inflammation as a common feature [2, 3]. Inflammatory markers are potential tools for distinguishing different asthma phenotypes and thereby enabling guidance in the choice of

treatment in a specific individual. An increasingly used marker of primarily T helper cell type 2 (TH₂)-driven inflammation in the airways is the fraction of nitric oxide in exhaled air (FeNO). FeNO is used as a marker [4] to follow the steroid-responsive inflammatory component of asthma. Guidelines for using FeNO in clinical practice [5] have been published by the American Thoracic Society (ATS) [6]. Some studies indicate that FeNO-guided treatment leads to improved asthma control and risk of asthma exacerbations [7–9], whereas other studies failed to confirm this [10]. Blood eosinophil counts and eosinophil cationic protein levels in serum (S-ECP) are probably influenced by non-pulmonary triggers in patients with asthma, while FeNO is primarily influenced by local TH₂-cytokine mechanisms in the airway mucosa [11]. ECP is a protein contained in the granules from the eosinophils and has high correlation with blood eosinophil counts [12, 13]. Elevated blood eosinophil count and S-ECP are linked to an increased rate of asthma exacerbations and increased symptoms [14, 15].

We have recently reported that a combination of FeNO and measures of systemic eosinophil inflammation has additive value in relation to asthma symptoms and asthma-related emergency room visits in a population-based study [15]. This has also been shown among adolescents and young adults from a clinical asthma population [16]. The aim of this study was to investigate whether simultaneously increased systemic eosinophil inflammation, assessed through S-ECP and local airway inflammation, assessed through elevated levels of FeNO, is related to an additive effect in the association with asthma attacks and symptoms than each of these markers taken individually when comparing with the group with normal levels in an unselected asthma population.

Method

Study population

The study is based on the Swedish part of the Global Allergy and Asthma European Network (GA2LEN) survey, conducted in 19 European centres, with the aim to study asthma, rhinitis and chronic rhinosinusitis (CRS). The first step of the survey was a random population-based sample among those aged 16–75 years. The selected subjects received a postal survey in 2008. In Sweden about 60% (27 866 individuals) responded, after up to three reminders were mailed. From among the responders, four subgroups were selected (participants with chronic rhinosinusitis (CRS), asthma and CRS, asthma, and controls) and invited for a clinical follow-up [17]. The participants were examined with lung function tests, skin prick tests and questionnaires about asthma symptoms, asthma attacks and

medication. A total of 1329 individuals participated in the clinical follow-up in Sweden, of whom 604 identified as asthmatics. An extension of the study with collections of extra blood samples for measuring S-ECP and measurements of FeNO was carried out in three of the Swedish centres: Stockholm, Uppsala and Umeå. The participants from Gothenburg (151 individuals, 105 with asthma) were therefore not included. Participants reporting physician-diagnosed chronic obstructive pulmonary disease (COPD) ($n = 84$) were excluded from the studied group. Subjects lacking FeNO ($n = 13$) or S-ECP ($n = 63$) measurements were excluded resulting in a study population of 339 individuals (Fig. 1).

Asthma symptoms, asthma exacerbations and asthma medication

Asthma was defined as self-reported diagnosis of asthma and either asthma symptoms in the past 12 months – wheezing, attack of shortness of breath and/or awakening at night with breathlessness – or asthma treatment (taking any medicines to help breathing at any time in the past 12 months) [17]. Participants with a history of asthma were asked whether they had experienced asthma attacks in the last 12 months, and if so, how many. In the group with attacks during the past 12 months, each participant was asked whether he or she had experienced any attacks in the last

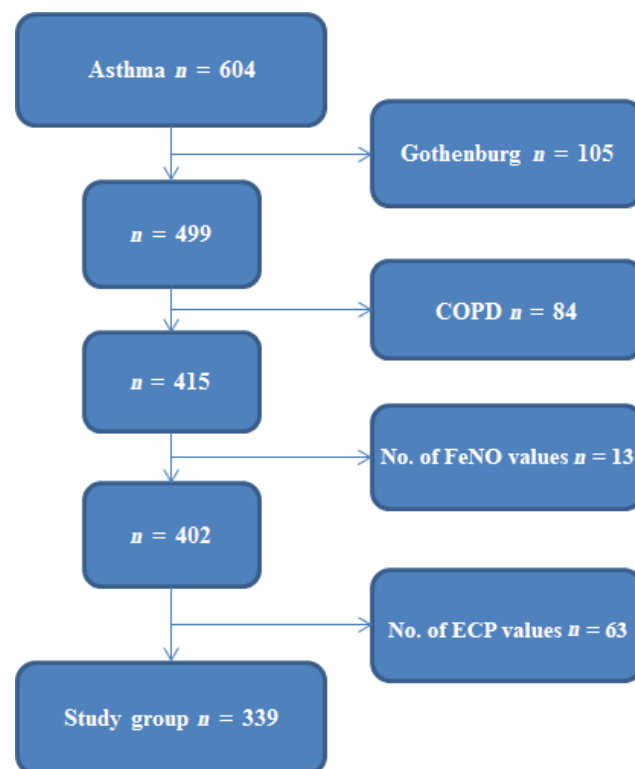


Fig. 1. Flow chart of included population.

3 months and if so, how many. The questions concerning asthma symptoms were based on the European Community Respiratory Health Survey [18] and were asked of each participant by a clinical interviewer. Information on use of asthma medication: long/short-acting beta agonists (LABA/SABA), inhalation corticosteroids (ICS) and anti-muscarinic inhalations in the past 12 months and the past week, as well as the use of oral corticosteroids and leukotriene receptor antagonists the past year, was also obtained in the structured interviews. Asthma onset after 18 years of age was defined as 'adult onset asthma'.

Lung function

Lung function assessment was performed in accordance with the ATS standards, using the EasyOne Spirometer (nnd Medizintechnik AG). Forced vital capacity (FVC) and forced expiratory volume in 1 s (FEV_1) were registered before and 15 min after bronchodilation with 200 µg salbutamol. Post-dilatation FEV_1 /FVC, FEV_1 and FVC were analysed. For predicted FVC and FEV_1 , Hedenström [19, 20] reference values were used, while the Hankinson reference values [21] were used for lower limits of normal (LLN) for the FEV_1 /FVC ratio.

Body mass index

Body mass index was calculated by dividing weight (in kilograms) with squared length (expressed in metres).

Skin prick test

A positive skin prick test (SPT) was defined as a wheal at least 3 mm at the widest diameter [17]. Atopy was defined as the presence of at least one positive SPT finding. SPT was performed with timothy grass, mixed grass, *Dermatophagoides pteronyssinus*, cat, dog, birch, cockroach, olive, *Alternaria*, *Artemisia*, *Parietaria* and *Dermatophagoides farina*.

FeNO

Fraction of exhaled NO (FeNO) was assessed in accordance with the recommendations from the ATS and European Respiratory Society [22] using a hand-held device with electrochemical sensor, NIOX MINO (NIOX MINO; Aerocrine, Stockholm, Sweden). A FeNO value ≥ 25 ppb was defined as elevated, in accordance with the ATS clinical guidelines [6].

S-ECP

Blood serum samples used for ECP analysis were collected in SSTs and left for 60 min at room temperature.

Thereafter, they were centrifuged at 3000 rpm for 15 min and stored in Sarstedt sample tubes at -20°C . The samples were analysed with a standard enzyme radioimmunoassay (ImmunoCAP, Thermo Fisher Scientific, Immunodiagnosics, Uppsala, Sweden). An S-ECP level ≥ 20 µg/L was defined as elevated [13, 23, 24].

Smoking history

The participants were divided into three groups: current smokers, previous smokers or never smokers. Previous smokers were defined as having smoked an amount of at least 20 packages of cigarettes or 360 g of tobacco per year, or one cigar a week, for at least one year. Current smokers were defined as currently smoking at least the same amount. Participants who had stopped smoking during the month previous to the study were also regarded as current smokers.

Statistical analyses

The statistical analyses were performed with Stata/IC 13.1 (Stata Corp, College Station, TX, USA) and the graphs with GraphPad Prism 6.07 for Windows (GraphPad Software, La Jolla, CA, USA). Chi-squared tests were performed for differences in prevalence of asthma symptoms and asthma attacks (as well as the other categorical variables) between the group with normal S-ECP and FeNO levels and the groups with either elevated S-ECP or elevated FeNO or both elevated S-ECP and elevated FeNO. Between-group differences of continuous variables, age and BMI were tested using unpaired t-tests. Multiple logistic regression, with the outcome variables asthma symptoms or asthma attacks (during the last 12 months, and during the 3 months prior to the study), was used to estimate the relation between FeNO and ECP categories. These models were adjusted for age, sex, BMI, smoking history, study centre atopy and medication (long-acting beta agonist (LABA) use the previous week, inhaled corticosteroids (ICS) previous week, leukotriene receptor antagonists (LTRA) the previous year and oral corticosteroids the previous year). Interaction analysis between age groups and gender was carried out with an adjusted logistic regression, adjusted for age, sex, BMI, smoking history, study centre atopy and medication (long-acting beta agonist (LABA) use the previous week, inhaled corticosteroids (ICS) previous week, leukotriene receptor antagonists (LTRA) the previous year and oral corticosteroids the previous year). Interaction analysis between elevated FeNO and elevated S-ECP was also carried out to evaluate the independent and synergistic effects of the markers.

To evaluate the sensitivity and consistency of the analysis, lower (15 µg/mL and 18 ppb, median values

in the material) and higher (26 µg/mL and 34 ppb, the limits for the upper quintiles) cut-offs for the definition of elevated ECP and FeNO were analysed. A P -value < 0.05 was considered to be statistically significant. Missing data were handled through complete cases.

Ethics statement

The study was approved by the Regional Ethical Review Board in Stockholm, Sweden (Dnr. 2008/1100-31/4), for all participating centres, and the collected personal data were treated in accordance with the Swedish personal data act.

Results

Study population

A total of 339 subjects were included in the present analysis (Table 1). Almost half of them, 164 (48%), had normal S-ECP and FeNO values, 63 (19%) had elevated S-ECP, 67 (20%) had elevated FeNO, and 45 (13%) had both elevated S-ECP and elevated FeNO. The group with adult asthma onset constituted 44% ($n = 149$) of the study group. The Pearson's correlation coefficient between (log-transformed) S-ECP and FeNO levels was 0.15 ($P = 0.007$) (Fig. S1).

Differences in subject characteristics between included and excluded subjects

The participants excluded due to lack of ECP and/or FeNO measurements ($n = 76$) were less likely to be atopic than subjects in the included group ($P = 0.002$). No differences in anthropometric characteristics or lung function were found between groups (data not shown). The excluded group also had a higher prevalence of asthma symptoms at rest ($P = 0.02$) and nocturnal

attacks of breathlessness ($P = 0.004$). No differences in the prevalence of self-reported asthma attacks during last 3 months ($P = 0.50$) and 12 months ($P = 0.74$) were found (data not shown).

Differences in subject characteristics with regard to S-ECP and FeNO levels

There were no differences in age, BMI, lung function or prevalence of adult asthma onset between subjects, divided according to the S-ECP and FeNO groups (Table 2). The group with normal S-ECP and normal FeNO levels had a higher prevalence of women than the group with elevated S-ECP and normal FeNO ($P = 0.009$), while fewer women were found in the normal S-ECP and elevated FeNO group. The normal S-ECP and FeNO value group had more smokers and fewer subjects with atopy than the elevated S-ECP and elevated FeNO group (Table 2). The use of LABA was higher in the elevated S-ECP and normal FeNO group; otherwise, there were no differences between the groups in medication use.

Asthma symptoms and attacks

The prevalence rates of wheeze, wheeze without having a cold and attacks of breathlessness after physical exercise were significantly higher in participants with elevated ECP and elevated FeNO, compared with participants with normal S-ECP and normal FeNO levels (Table 3). The elevated ECP and elevated FeNO group also had a significantly higher prevalence of asthma attacks in the last 3 and 12 months (Table 3).

These associations were consistent after adjustment for sex, BMI, age, study centre, smoking history, atopy and medication use (long-acting beta agonists the previous week, inhalation of steroids the previous week, LTRA last year and oral corticosteroids the last year) (Figs 2 and 3). In the adjusted model, singly elevated S-ECP was closer related to asthma attacks with higher ORs than singly elevated FeNO. In contrast, singly elevated FeNO related closer to asthma symptoms than singly elevated S-ECP, while the simultaneously elevated markers had a significant association with both symptoms and attacks (Figs 2 and 3). No significant interactions were found between elevated S-ECP and FeNO levels towards any of the outcome variables ($P_{\text{interaction}} > 0.1$) (Table 4). There was no difference in the association between having elevated S-ECP and elevated FeNO and asthma symptoms or attacks with relation to gender or age ($P_{\text{interaction}} > 0.1$) (Table S1).

Some associations with singly elevated biomarkers were also seen. Asthma attacks during the last 3 months (aOR 2.4, CI: 1.1–4.4) were more likely in the elevated S-ECP and normal FeNO group and in the

Table 1. Characteristics of study population

Age (median (range))	40 (17–75)
Female sex (N (%))	200 (59%)
Atopy (N (%))	246 (76%)
Asthma onset < 18 years (N (%))	190 (56%)
Smoke history	
Never smoker (N (%))	206 (61%)
Ex-smoker (N (%))	111 (33%)
Current smoker (N (%))	22 (6.5%)
BMI	
< 18.5 kg/m ² (N (%))	5 (1.5%)
18.5–25 kg/m ² (N (%))	153 (45%)
25–30 kg/m ² (N (%))	112 (33%)
≥ 30 kg/m ² (N (%))	69 (20%)

Missing data ($n =$ missing number): atopy $n = 16$.

Table 2. Characteristics of the population divided by FeNO and S-ECP levels. Groupwise comparisons between normal S-ECP and normal FeNO group and the groups with elevated inflammatory markers (mean $\pm$ SD)

Per cent of the subgroups (listed below) divided by inflammatory pattern	FeNO normal S-ECP normal (n = 116)	FeNO normal S-ECP elevated (n = 63)	P-value, prevalence	FeNO elevated S-ECP normal (EF) (n = 67)	P-value, prevalence	FeNO elevated S-ECP elevated (n = 45)	P-value, prevalence
Age	42 $\pm$ 14	39 $\pm$ 14	0.51	46 $\pm$ 14	0.34	38 $\pm$ 13	0.57
Women	61%	79%	0.009	35%	0.000	60%	0.93
Atopy	69%	77%	0.26	82%	0.053	93%	0.001
Adult onset asthma	46%	46%	0.97	46%	0.94	31%	0.079
BMI	26 $\pm$ 4.5	27 $\pm$ 6.2	0.36	26 $\pm$ 3.9	1.0	26 $\pm$ 4.8	1.0
Smoking history			0.41		0.38		0.043
Non-smoker	60%	62%		51%		78%	
Ex-smoker	34%	27%		43%		22%	
Current smoker	6.7%	11%		6.0%		0%	
FEV ₁ /FVC < LLN	4.6%	7.0%	0.48	3.1%	0.63	4.8%	0.95
FEV ₁ % < 80% pred.	19%	21%	0.78	16%	0.67	13%	0.34
FVC < 80% pred.	16%	16%	0.60	17%	0.86	17%	0.95

Missing data (n = missing number): atopy n = 16, FEV₁/FVC n = 22, FVC n = 22, FEV₁ n = 27.

Table 3. The proportion of symptomatic individuals in the population divided into groups depending on S-ECP and FeNO levels. Groupwise comparisons of the distribution between the normal S-ECP/normal FeNO levels group and the groups with elevated inflammatory markers

Proportion of symptomatic individuals (in per cent) in the groups, respectively	S-ECP normal FeNO normal (n = 116, %)	S-ECP elevated FeNO normal (n = 63, %)	P-value	S-ECP normal FeNO elevated (n = 67, %)	P-value	S-ECP elevated FeNO elevated (n = 45, %)	P-value
Wheeze	80	75	0.33	87	0.27	93	0.04
Wheeze when breathless	55	63	0.27	63	0.32	69	0.11
Wheeze when not having a cold	53	57	0.61	70	0.02	78	0.003
Nocturnal chest tightness	40	38	0.77	45	0.53	40	0.98
Attacks of breathlessness at rest	17	11	0.27	16	0.90	20	0.65
Breathlessness after physical exercise	31	37	0.44	42	0.12	51	0.01
Nocturnal attacks of breathlessness	20	17	0.54	21	0.89	16	0.47
Asthma attacks past 12 months	43	48	0.56	39	0.56	60	0.047
Asthma attack past 3 months	25	35	0.14	31	0.34	51	0.001

Missing data (n = missing number): Wheeze when not having a cold n = 1, nocturnal attacks of breathlessness n = 6, asthma attacks past 12 months n = 3, asthma attack past 3 months n = 3.

normal S-ECP and elevated FeNO group attacks of breathlessness after physical exercise were more likely (aOR 2.0, CI: 1.1–3.9), both compared with the normal S-ECP/normal FeNO level group (Figs 2 and 3).

There were more individuals who had experienced asthma symptoms (breathlessness after exercise) in the elevated ECP and elevated FeNO group vs. in the elevated S-ECP and normal FeNO group (aOR: 4.0 CI: 1.6–9.8) and more who had asthma attacks the past 3 and 12 months in the elevated S-ECP and elevated FeNO group vs. the normal S-ECP and elevated FeNO group (OR: 2.9 CI: 1.2–6.8) (data not shown).

Elevated S-ECP was independently related to more asthma attacks after adjustment for elevated FeNO,

while elevated FeNO was independently associated to more symptoms after exercise (Table 4). No significant interactions between having elevated FeNO and elevated ECP on the effect on different asthma outcomes were found (Table 4).

Using lower cut-offs, the associations with asthma symptoms or attacks in most of the cases became weaker and, not statistically significant (Table S2). Raising the cut-off levels resulted in a weaker correlation between singly elevated S-ECP and asthma attacks and a stronger correlation between singly elevated FeNO and asthma symptoms. The simultaneously elevated markers related to higher odds ratios both for symptoms and attacks the last year (Table S2).

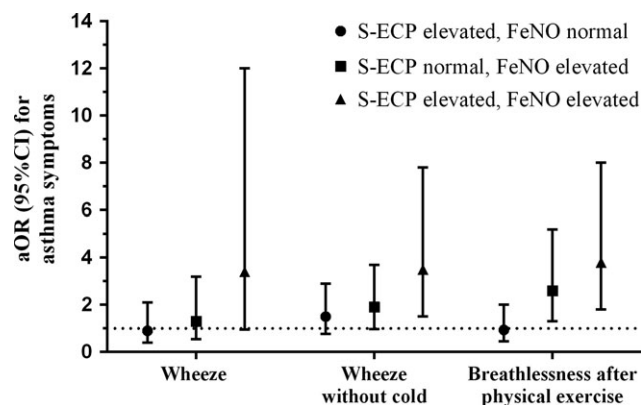


Fig. 2. Adjusted* odds ratios (aOR) (95% CI) of having asthma symptoms in relation to levels of FeNO and S-ECP (compared with having normal levels of FeNO and S-ECP). *Adjusted for BMI, age, sex, study centre, smoking history, atopy, OCS use last 12 months, LABA use last week, ICS use last week and LTRA use last year.

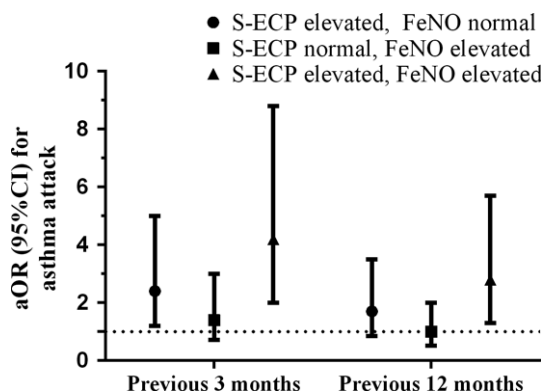


Fig. 3. Adjusted* odds ratios (aOR) (95% CI) of having asthma attacks in relation to levels of FeNO and S-ECP (compared with having normal levels of FeNO and S-ECP). *Adjusted for BMI, age, sex, study centre, smoking history, atopy, OCS use last 12 months, LABA use last week, ICS use last week and LTRA use last year.

Discussion

The main finding of this study is that simultaneously elevated S-ECP and elevated FeNO levels relate to a higher prevalence of recent asthma attacks and asthma symptoms. These findings appeared not to be highly influenced by atopy, gender, age or adult asthma onset.

Individually, the correlation between either elevated S-ECP or elevated FeNO and symptoms and asthma attacks were weaker, and singly elevated S-ECP levels related to asthma attacks in the last 3 months, while singly elevated FeNO levels related to attacks of breathlessness after physical exercise. Similarly, it appears that FeNO is strongly related to previous symptoms while S-ECP is strongly related to a previous history of exacerbations when both markers are used as independent predictors of asthma-related outcomes.

Simultaneously elevated S-ECP and FeNO related to increased likelihood of recent asthma attacks and asthma symptoms. The blood eosinophil count and S-ECP are strongly dependent on IL-5 [25, 26]. Treatment with IL-5-blocking monoclonal antibodies is highly efficient in reducing blood eosinophil levels and exacerbations but does not affect FeNO [25, 26]. FeNO on the other hand is strongly dependent on IL-4 and IL-13 pathways, and treatment with monoclonal antibodies against IL-4 significantly lowers FeNO levels and has been shown to decrease sensitivity to allergen exposure [27], increase lung function and reduce symptoms [28]. Monoclonal blocking of IL-13 shows a similar decrease of FeNO levels, but does not affect blood eosinophil levels [29, 30]. Elevated S-ECP or blood eosinophil levels have been linked to severe asthma attacks [14, 15, 25, 26], while FeNO represents local inflammation in the airways and relates closely to bronchial hyperresponsiveness [15, 27, 28, 31, 32]. This is in line with our finding in a population-based study [15] and also a study with asthmatic adolescents [16] and supports that elevated S-ECP and FeNO levels signal for different inflammatory pathways [11, 33]. Similar results with regard to asthma control have been reported by combining another marker of local airway inflammation: sputum and blood eosinophils [34]. Finally, a recent study reported that the response to treatment was better predicted by combining several markers of inflammation, such as FeNO, urinary bromotyrosine levels and sputum eosinophil counts [35].

The associations between exacerbations, symptoms and elevated FeNO and elevated S-ECP were consistent in both atopic and non-atopic individuals and were valid after adjustment for gender, age and adult onset of

Table 4. The association between elevated S-ECP and elevated FeNO to asthma events after adjustment for FeNO and S-ECP (adjusted* odds ratio (95% confidence interval))

Symptom	S-ECP elevated	P-value	FeNO elevated	P-value	Interaction term	P _{interaction}
Wheeze	0.92 (0.41–2.1)	0.84	1.3 (0.54–3.2)	0.55	2.8 (0.56–14)	0.21
Wheeze when not having a cold	1.5 (0.77–2.9)	0.23	1.9 (0.97–3.7)	0.061	1.2 (0.40–3.7)	0.72
Breathlessness after physical exercise	0.95 (0.45–2.0)	0.88	2.6 (1.3–5.2)	0.006	1.5 (0.51–4.5)	0.45
Attack past 12 months	1.7 (0.88–3.5)	0.11	1.0 (0.51–2.0)	0.98	1.6 (0.54–4.5)	0.40
Attack past 3 months	2.4 (1.2–5.0)	0.015	1.4 (0.71–3.0)	0.31	1.2 (0.40–3.5)	0.77

*Adjusted for BMI, age, sex, study centre, smoke history, atopy, OCS use past 12 months, LABA use past week, ICS use past week, LTRA use past year and FeNO or S-ECP as categorical variables.

asthma. This has not been studied specifically in other studies, but suggests that this combination of biomarkers is robust for different eosinophil asthma phenotypes. Also, in this material, no relation to lung function was found. This further indicates the additive value in detecting active inflammation through inflammatory markers.

This study has evaluated a large group of asthmatic individuals, but has some limitations. The investigation studied the risk of symptoms and attacks retrospectively, which impairs the ability to draw conclusions about the predictive value of the markers when studied prospectively. The study period of one year preceding the data collection also makes the influence of confounding factors such as corticosteroid use hard to evaluate, as the causal relationships are not possible to trace in detail. There is also a risk for recall bias, which could influence the results. However, the associations between inflammation and attacks tended to be more pronounced during the 3 months before data collection than 12 months before, indicating a true association. The cut-off value at 25 ppb for elevated FeNO can be discussed. FeNO can be influenced by a range of constitutional parameters, such as height, age and gender [36–38], the latter being a likely reason for the low prevalence of women in the group with elevated FeNO values and the higher prevalence of asthma events in this group. However, a FeNO level of 25 ppb is often used as the limit between low and intermediate levels [6], and levels above this value indicate an increased inflammatory activity [5]. No significant interaction was found between elevated FeNO and elevated S-ECP towards any of the outcome variables. This indicates that the combined effect of the two studied inflammatory markers was additive but not synergistic.

The levels of both FeNO and S-ECP are influenced by smoking [12, 24, 39, 40], although in different directions, which could be an issue when applying this matrix in currently smoking asthmatics. However, the number of currently smoking subjects in this material was low, and the main results were adjusted for smoking.

In conclusion, having simultaneously elevated FeNO and S-ECP levels relates to a higher prevalence of recent asthma attacks and asthma symptoms as compared with normal levels. These results support the hypothesis that FeNO and S-ECP are markers for different inflammatory

pathways in asthma and that the combined use of these two markers may be a helpful tool for both achieving a better understanding of the pathogenesis in asthma and for better asthma control in eosinophil asthma. Further, elevated FeNO was closer related to asthma symptoms, while elevated S-ECP related to exacerbations. This might indicate different effects on morbidity patterns and that a successful treatment should target to have both inflammatory markers and pathways under control. The results should be confirmed in a clinical population with a prospective study design.

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Conflict of interest

Magnus P. Borres is an employee of Thermo Fisher Scientific, Immunodiagnostics, and participated in the study as adjunct professor at Uppsala University. Kjell Alving is a former employee at Aerocrine AB and has received consultancy fees from Aerocrine AB (producer of exhaled NO devices). None of the funding agencies or industry partners had any role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author contributions

IM, CJ and AM analysed the data. IM and AM wrote the first draft of the manuscript. SED, CJ and AM supervised the study and contributed to the design of present data analyses. AB, JS, GH, KA and MB contributed to drafting the manuscript, and all co-authors revised it critically.

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Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Correlation between S-ECP and FeNO.

Table S1. Adjusted* odds ratios for reporting asthmatic symptoms and asthma attacks when having

simultaneously elevated FeNO and elevated S-ECP (compared with normal FeNO and normal S-ECP), dividing subjects according to gender or age.

Table S2. Adjusted Odds Ratio to experienced asthma events depending on inflammatory pattern, different cut offs in relation to respectively normal values.