Original Article

Performance of Eosinophil Cationic Protein as a Biomarker in Asthmatic Children

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What is already known about this topic? Blood eosinophils are a frequently used marker of type 2 inflammation in children with asthma, but their sensitivity for predicting outcomes is relatively poor. Alternative markers of type 2 inflammation may be beneficial in children.

What does this article add to our knowledge? Plasma eosinophil cationic protein (ECP) concentrations were associated with type 2 inflammatory markers, poorer asthma control and lung function, and historical exacerbations. Higher plasma ECP also predicted systemic corticosteroid responsiveness and future exacerbations at 12 months.

How does this study impact current management guidelines? Plasma ECP may be a useful marker of type 2 inflammation and may help identify those children at highest risk for recurrent exacerbations who could benefit from corticosteroid treatment.

BACKGROUND: Although blood eosinophils are a frequently used marker of type 2 inflammation in children with asthma, their sensitivity is relatively poor. Additional markers of type 2 inflammation are needed.

OBJECTIVE: We hypothesized that plasma concentrations of eosinophil cationic protein (ECP), a marker of eosinophil activation, would be useful for detection of type 2 inflammation and would predict poorer asthma outcomes over 1 year.

METHODS: Children and adolescents 6 through 17 years (N = 256) with confirmed asthma completed a baseline visit and a follow-up visit at 12 months. A subset also underwent systemic corticosteroid responsiveness testing with intramuscular triamcinolone. Outcome measures at 12 months included uncontrolled asthma, lung function, and asthma exacerbations treated with systemic corticosteroids.

RESULTS: Plasma ECP concentrations ranged from 0.03 to 413.61 ng/mL (median, 6.95 ng/mL) and were consistently associated with other markers of type 2 inflammation. At baseline, children in the highest ECP tertile had poorer asthma control, more airflow limitation, and more exacerbations, but also had greater symptom improvement with intramuscular triamcinolone. At 12 months, associations between the highest ECP tertile and exacerbations, but not lung function or asthma control, persisted after covariate adjustment. However, the sensitivity of ECP was modest and was not markedly different from that of blood eosinophil counts.

CONCLUSION: Plasma ECP concentrations may be a useful marker of type 2 inflammation in children and may help identify those children at highest risk for recurrent exacerbations who could benefit from corticosteroid treatment. However, additional markers may be needed to improve sensitivity for outcome detection. © **2021 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2021;■:■-■)**

Key words: *Asthma in children; Eosinophils; Exacerbation; Type 2 inflammation*

Type 2 inflammation, which involves the recruitment and activation of eosinophils by Th2 cytokines and other pathways, is thought to be a pivotal risk factor for persistent wheezing and poor asthma control in children.[1] Children with type 2 inflammation, compared with those with non−type 2 inflammation, often have increased symptoms, impaired lung function measures with increased airway hyperresponsiveness, and more frequent exacerbations.[2-5] This type 2 "high" asthma phenotype also tends to be more corticosteroid responsive.[3-6] Therefore, identification of children with type 2 "high" asthma in the clinical setting may be useful for selection of the most appropriate asthma controller medications. However, there is no consensus regarding the "best" biomarker for type 2 inflammation, or the appropriate cut-points in pediatric populations.[7,8]

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Abbreviations used
ACQ- Asthma Control Questionnaire
ACT- Asthma Control Test
CACT- Childhood Asthma Control Test
CCL- CC chemokine ligand
ECP- Eosinophil cationic protein
FEF_{25-75}- Forced expiratory flow at 25%-75% of vital capacity
FEV_1- Forced expiratory volume in 1 second
FVC- Forced vital capacity
PAQLQ- Pediatric Asthma Quality of Life Questionnaire

The blood eosinophil count is currently the most accepted biomarker of type 2 inflammation in routine clinical practice, but there are also challenges with its measurement. After eosinophils mature in the bone marrow, they are present in the circulation for 8 to 12 hours before migrating to tissue, where they remain for 7 to 14 days.[9] In contrast, eosinophil cationic protein (ECP) is an eosinophil granule protein that is primarily released after the eosinophil has left the circulation,[10] with a turnover time in the blood of approximately 45 minutes.[11] ECP is therefore considered a marker of eosinophil activation[12] and, like blood eosinophils, has been associated with several clinical features of atopic asthma.[13]

Although ECP has been of interest in the asthma field for several decades, nearly all the existing studies of ECP in children are cross-sectional in nature and have employed either a case/control design or correlative analyses.[13] In the few clinical trials that examined ECP as a predictor of treatment response, ECP concentrations were measured at only a single time point.[5,14] Other clinical trials of asthma controller medications or longitudinal studies that measured ECP before and after treatment were limited to very small samples of children ($n < 35$) with mild asthma.[15-18] Phenotyping in these studies was also quite limited. Therefore, major gaps related to the clinical utility of ECP in real-world pediatric asthma populations still persist.

We questioned whether plasma ECP concentrations would be a useful biomarker of type 2 inflammation at multiple time points and whether plasma ECP concentrations could discriminate clinical outcomes in children with asthma. We hypothesized that: (1) ECP concentrations in children with asthma would correlate with blood eosinophil counts, the magnitude of aeroallergen sensitization, serum IgE levels, exhaled nitric oxide concentrations, and plasma concentrations of IL-4, IL-5, IL-13, and CC chemokine ligand (CCL)-11 (eotaxin); and (2) higher ECP concentrations would be associated with poorer asthma control, greater airflow limitation, and a greater occurrence of exacerbations over 12 months of follow-up. We further hypothesized that children with higher ECP concentrations would have a greater response to treatment with systemic corticosteroids.

METHODS

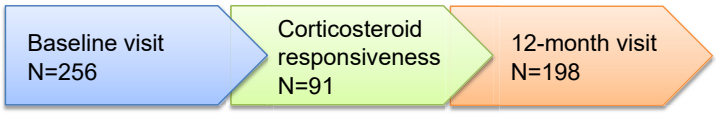
Children 6 through 17 years of age with physician-diagnosed atopic asthma across the severity spectrum treated with controller medications for at least 8 weeks were eligible for the study if they had: (1) current or historical evidence of $\geq$12% reversibility in their forced expiratory volume in 1 second (FEV_1) relative to baseline after bronchodilator administration or (2) airway hyperresponsiveness to methacholine, with a provocative concentration of methacholine causing a 20% drop in FEV_1 of $\leq$16 mg/mL. Atopy was defined as either sensitization to at least 1 aeroallergen, blood eosinophil counts >300 cells/μL, or physician-diagnosed allergic rhinitis or atopic dermatitis. Exclusion criteria included premature birth before 35 weeks of gestation and other chronic airway disorders that could mimic asthma such as pulmonary aspiration or vocal cord dysfunction. Permission to proceed with this study was granted by the Emory University Institutional Review Board. Informed written consent was obtained from legal guardians. Verbal assent was obtained from children 6 to 10 years of age, and written assent was obtained from children and adolescents 11 to 17 years.

Study design and outcome measures

Participants completed a baseline outpatient research visit and a follow-up outpatient research visit at 12 months. Research visits were scheduled between 9 am and 2 pm on weekdays. These visits were postponed if an asthma exacerbation treated with systemic corticosteroids was reported within the preceding 2 weeks. After the baseline visit, a subset of children also underwent systemic corticosteroid responsiveness testing with intramuscular triamcinolone acetonide (1 mg/kg, 60 mg maximum dose) administered in the gluteal muscle. Corticosteroid responsiveness was assessed at 14 days (window +7 days) after triamcinolone receipt and was quantified by asthma control and lung function measures. Outcome measures at 12 months included uncontrolled asthma at 12 months, lung function at 12 months, and the occurrence of any asthma exacerbation treated with systemic corticosteroids in the prior 12 months.[19]

Procedures

The schedule of procedures is shown in Figure 1. Participants and their caregivers completed questionnaires pertaining to symptoms, medical history, and demographics. Asthma control was assessed with the 6-item Asthma Control Questionnaire (ACQ)[20] administered by a trained interviewer.[21] Participants also completed the Asthma Control Test (ACT)[22] or the Childhood Asthma Control Test (CACT).[23] Asthma-related quality of life was assessed with the Pediatric Asthma Quality of Life Questionnaire (PAQLQ)[24] with technical assistance recommended for younger children,[25] where higher PAQLQ scores reflect better quality of life. Exhaled nitric oxide concentrations were measured according to technical standards[26] with a commercial device (NIOX MINO; Circassia Pharmaceuticals, Chicago, Ill). Aeroallergen sensitization was assessed by specific IgE testing by the ImmunoCAP method by Phadia (Children's Healthcare of Atlanta, Atlanta, Ga) or skin prick testing with 12 extracts: tree mix, grass mix, weed mix, *Ambrosia artemisiifolia*, *Alternaria alternata, Aspergillus fumigatus*, *Cladosporium herbarum*, dog dander, cat dander, *Blattella germanica*, *Dermatophagoides farinae,* and *Dermatophagoides pteronyssinus* (Greer Laboratories, Lenoir, NC). Venipuncture was also performed for quantification of blood eosinophils (Children's Healthcare of Atlanta) and total serum IgE (Children's Healthcare of Atlanta). Spirometry (KoKo PDS; Ferraris, Louisville, Colo) was performed according to technical standards[27] with 360 mcg albuterol sulfate for bronchodilator reversibility testing. The best of 3 forced vital capacity (FVC) maneuvers were interpreted according to Global Lung Function Initiative prediction equations.[28] FVC, FEV_1, FEV_1/FVC, and the forced expiratory flow at 25%-75% of vital capacity (FEF_{25-75}) were expressed as percentage of predicted values. A subset of participants also underwent bronchoprovocation testing with methacholine concentrations of 0 to 16 mg/mL (Provocholine; Methapharm Inc, Coral Springs, Fla) delivered by a Rosenthal



Procedure	Low ECP, N=85 Intermediate ECP, N=85 High ECP, N=86	Low ECP, N=31 Intermediate ECP, N=24 High ECP, N=36	Low ECP, N=69 Intermediate ECP, N=61 High ECP, N=68
ACQ	X	X	X
ACT/CACT	X		X
PAQLQ	X	X	X
Exhaled nitric oxide	X	X	X
Spirometry	X	X	X
Methacholine challenge	X		
Allergen sensitization	X		
Total IgE	X		
Blood eosinophils	X	X	X
Plasma ECP	X	X	X

FIGURE 1. Inclusion of participants and procedures performed at each visit. *ACQ*, Asthma Control Questionnaire; *ACT*, Asthma Control Test; *CACT*, Childhood Asthma Control Test; *ECP*, eosinophil cationic protein; *PAQLQ*, Pediatric Asthma Quality of Life Questionnaire.

dosimeter (Pulmonary Data Service Instrumentation, Louisville, Colo). Bronchoprovocation was limited to participants with baseline FEV_1 >70% predicted.

ECP was quantified in plasma using a commercially available kit (TSZ Biological catalog #HU8604, San Francisco, Calif). Cytokines and chemokines were quantified in the plasma with multiplex assays for IL-4, IL-5, IL-13, and CCL-11/eotaxin according to technical standards (#HSTCMAG-28SK and #HCYTOMAG-60K; MilliporeSigma, Burlington, Mass).

Statistical analyses

Data were analyzed with SPSS Statistics (Version 26; IBM, Armonk, NY). Data that were not normally distributed were logarithmically transformed before analyses. ECP tertiles were compared with χ^2 tests and analysis of variance, with Tukey's least significant difference tests for *post hoc* comparisons. Linear and logistic regression analyses were performed with covariate adjustment as indicated. Receiver operating characteristic curves were generated to evaluate sensitivity and specificity. A P value of <.05 was used as the threshold for statistical significance without adjustment for multiple comparisons.

RESULTS

Two hundred fifty-six children were enrolled and completed the baseline characterization visit (Figure 1). Their features are shown in Table I. Plasma ECP concentrations ranged from 0.03 to 413.61 ng/mL (median, 6.95 ng/mL; 25-75th percentile, 1.81-23.75 ng/mL). ECP concentrations did not differ by age, duration of asthma, ethnicity, obesity, or household tobacco smoke exposure. However, ECP concentrations did differ by sex, race, allergic rhinitis, and eczema (Figure E1, available in this article's Online Repository at www.jaci-inpractice.org). ECP concentrations were also significantly higher in children treated with high-dose inhaled corticosteroids (no inhaled corticosteroid treatment: median, 4.29 ng/mL, 25-75th percentile, 0.94-15.34 ng/mL; low-to-medium dose treatment: median, 5.88 ng/mL, 25-75th percentile, 1.33-20.07 ng/mL; high-dose treatment: 11.87 ng/mL, 25-75th percentile, 3.08-26.72 ng/mL; $P = .001$). The differences in ECP by sex, race, allergic rhinitis, and eczema persisted after adjustment for blood eosinophil counts, inhaled corticosteroid dose, and uncontrolled asthma (Table E1,

TABLE I. Features of the participants

Feature	All participants (N = 256)
Age (y)	10.5 (8.5, 13.83)
Asthma duration (y)	8.0 (5.3, 11.3)
Male	158 (61.7)
Hispanic ethnicity	3 (1.2)
Race	
White	28 (10.9)
Black	214 (83.6)
More than 1 race	14 (5.5)
Household education	
Refused	14 (5.5)
Did not complete high school	11 (4.3)
High school	39 (15.2)
Some college	89 (34.8)
College degree	103 (40.2)
Parent with asthma	152 (61.3)
Smoker in household	37 (14.7)
Other diagnoses	
Obesity	70 (27.3)
Allergic rhinitis	221 (87.4)
Atopic dermatitis	150 (59.3)
Asthma medications	
Inhaled corticosteroid (any dose)	213 (83.2)
High-dose inhaled corticosteroid	122 (47.7)
Leukotriene receptor antagonist	156 (60.9)
Long-acting β-agonist	133 (52.0)

Data represent the median (25th, 75th percentile) or the number of participants (%).

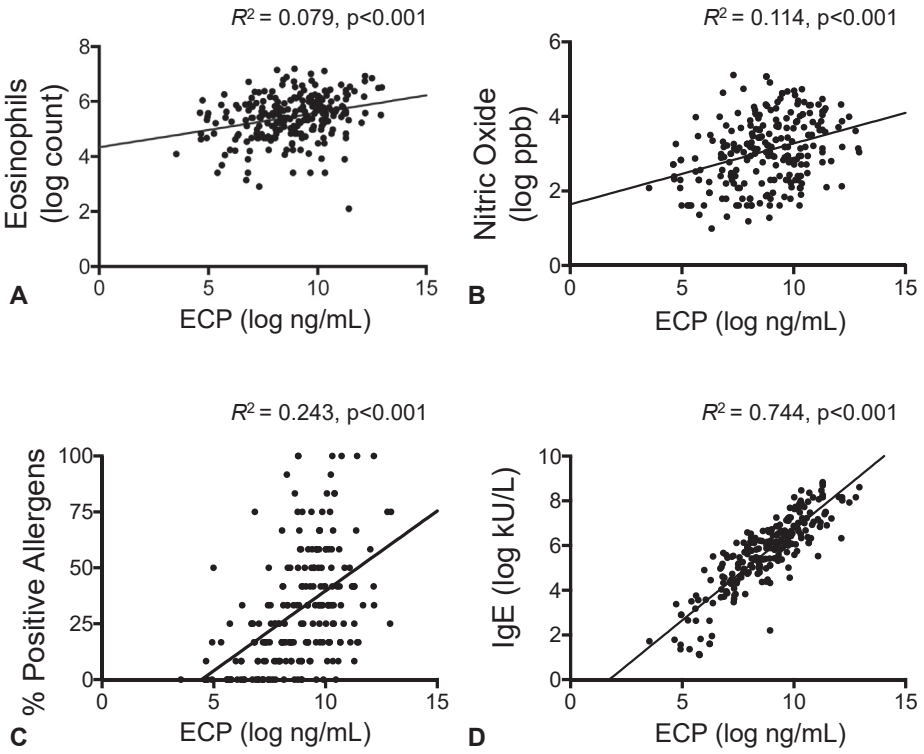


FIGURE 2. Associations between eosinophil cationic protein (ECP) concentrations and (**A**) blood eosinophil counts, (**B**) exhaled nitric oxide concentrations, (**C**) the percentage of positive aeroallergens, and (**D**) serum IgE concentrations.

available in this article's Online Repository at www.jaci-inpractice.org). ECP concentrations were also associated with other markers of type 2 inflammation, including blood eosinophils, exhaled nitric oxide, the percentage of positive aeroallergens, and serum IgE (Figure 2, *A-D*). There were no associations between plasma ECP concentrations and concentrations of plasma IL-4 ($r = 0.078$, $P = .236$), IL-5 ($r = -0.012$, $P = .861$), IL-13 ($r = 0.042$, $P = .528$), or CCL-11/eotaxin ($r = -0.027$, $P = .683$).

Associations between ECP and baseline asthma features

Because there are no established reference intervals of normal for plasma ECP, children were stratified into tertiles according to ECP concentrations as follows: Low ECP, <3.12 ng/mL; Intermediate ECP, 3.12-16.22 ng/mL; and High ECP, ≥16.23 ng/mL. Baseline asthma features associated with each ECP tertile are shown in Table II. Type 2 inflammatory features were elevated in both the Intermediate and High ECP groups, but the High ECP group was distinguished by a greater magnitude of aeroallergen sensitization. Also, both the Intermediate and High ECP groups had poorer asthma control, more airflow limitation, greater bronchodilator reversibility, and airway hyperresponsiveness compared with the Low ECP group. However, the High ECP group had significantly more exacerbations in the prior year (Table II).

Associations between ECP and corticosteroid responsiveness

Ninety-one children consented to treatment with intramuscular triamcinolone. Their features were not significantly different from the baseline sample (mean age, 12.2 ± 3.3 years; 66.7% male; 72.5% black). After triamcinolone treatment, ECP concentrations remained elevated in the High ECP group (Low ECP: 1.4 ± 0.9 ng/mL; Intermediate ECP: 14.9 ± 6.7 ng/mL; High ECP: 56.8 ± 7.3 ng/mL; $P < .001$). In contrast, blood eosinophil counts and exhaled nitric oxide concentrations were markedly reduced after triamcinolone irrespective of ECP grouping (Figure 3), and correlations between ECP, blood eosinophil counts, and exhaled nitric oxide were no longer present (ECP vs eosinophil count, $r = -0.074$, $P = .743$; ECP vs exhaled nitric oxide, $r = 0.268$, $P = .068$).

Asthma clinical outcomes after receipt of intramuscular triamcinolone are shown in Table III. Triamcinolone significantly improved (ie, reduced) ACQ-6 scores in the High ECP group, reflected by a larger mean difference in the ACQ-6 score from baseline, but did not significantly alter asthma quality of life or lung function measures.

Associations between ECP and outcomes at 1 year

One hundred ninety-eight participants (80%) provided outcome data at 12 months. Children lost to follow-up were more frequently black (98.3%) with higher household educational attainment (52.5% with college degree), less intense medication requirements (18.6% and 22.0% treated with high-dose inhaled corticosteroids and long-acting β-agonists, respectively), and better asthma control (37.3% with ACQ-6 score <1.50 or ACT or CACT score<20). Therefore, no attempts were made to impute the final dataset.

ECP concentrations, type 2 biomarkers, and asthma outcomes at 12 months are shown in Table IV. At 12 months, ECP concentrations remained elevated in the High ECP group. A

TABLE II. Baseline asthma and type 2 inflammatory features, by eosinophil cationic protein (ECP) tertile

Feature	Low ECP (<3.12 ng/mL) (N = 85)	Intermediate ECP (3.12-16.22 ng/mL) (N = 85)	High ECP (≥16.23 ng/mL) (N = 86)
Type 2 inflammatory markers			
Blood eosinophils (cells/μL)	230.1 ± 18.3	307.6 ± 27.4*	375.9 ± 26.8*
Blood eosinophils (%)	3.47 ± 0.31	5.25 ± 0.49*	6.23 ± 0.46*
Exhaled nitric oxide (ppb)	20.8 ± 2.9	36.5 ± 3.6*	36.9 ± 27.2*
Serum IgE (kU/L)	119.9 ± 14.8	426.8 ± 273.5*	1890.6 ± 169.2*^
% positive aeroallergens (of 12)	12.1 ± 1.8	35.0 ± 2.9*	43.5 ± 3.0*^
Exacerbation history (past year)			
Any systemic corticosteroid burst	52 (61.2)	48 (56.5)	65 (75.6)*^
Emergency department visit	38 (44.7)	45 (52.9)	52 (60.5)*
Hospitalization	21 (24.7)	19 (22.6)	24 (27.9)
Asthma control			
ACQ-6 score	0.96 ± 0.15	1.13 ± 0.14	1.14 ± 0.12
ACT or CACT score	20.5 ± 4.2	20.2 ± 4.0	18.8 ± 4.6
Uncontrolled asthma	32 (38.6)	45 (53.6)*	47 (54.7)*
PAQLQ score	5.57 ± 0.17	5.57 ± 0.16	5.50 ± 0.14
Lung function			
FVC (% predicted)	101.3 ± 1.4	102.4 ± 1.9	101.8 ± 1.8
FEV_1 (% predicted)	94.1 ± 1.4	90.9 ± 2.1*	88.3 ± 2.0*
FEV_1/FVC (% predicted)	92.6 ± 1.1	88.2 ± 1.3*	86.2 ± 1.2*
FEF_{25-75} (% predicted)	80.7 ± 2.9	71.4 ± 3.2*	66.7 ± 2.9*
Bronchodilator reversibility			
FEV_1 absolute reversibility (%)†	7.4 ± 1.0	11.8 ± 1.3*	13.0 ± 1.2*
FEV_1 relative reversibility (%)‡	8.2 ± 1.1	15.4 ± 2.0*	17.0 ± 1.8*
PC_{20} (mg/mL methacholine)§	14.9 ± 2.8	5.1 ± 1.6*	2.5 ± 1.0*

Data represent the mean ± standard error of the mean or the number of participants (%).
ACQ, Asthma Control Questionnaire; *ACT*, Asthma Control Test; *CACT*, Childhood Asthma Control Test; *FEF_{25-75}*, forced expiratory flow at 25%-75% of vital capacity; *FEV_1*, forced expiratory volume in 1 second; *FVC*, forced vital capacity; *PAQLQ*, Pediatric Asthma Quality of Life Questionnaire; *PC_{20}*, provocative concentration causing a 20% drop in FEV_1.
*$P < .05$ vs Low ECP; ^$P < .05$ vs Intermediate ECP.
†Defined as: % predicted $FEV_{1\ \text{post-bronchodilator}}$ − % predicted $FEV_{1\ \text{baseline}}$
‡Defined as: ($FEV_{1\ \text{post-bronchodilator}} - FEV_{1\ \text{baseline}}$)/$FEV_{1\ \text{baseline}} \times 100$
§Low ECP, n = 14; Intermediate ECP, n =19; High ECP, n = 23.

total of 85% of children in the Low ECP group had ECP concentrations that remained "low" (ie, <3.12 ng/mL), whereas 95% of children in the High ECP group had ECP concentrations that remained "high" (ie, >16.23 ng/mL). The Intermediate ECP group was less stable, with only 50% of children remaining intermediate; 19% had concentrations <3.12 ng/mL and 31% had concentrations >16.23 ng/mL. Similar to observations at baseline, at 12 months of follow-up, ECP concentrations were again correlated with blood eosinophil percentages ($r = 0.362$, $P = .010$), blood eosinophil counts ($r = 0.319$, $P = .024$), and exhaled nitric oxide values ($r = 0.294$, $P = .022$). Children in the High ECP group also had a higher prevalence of uncontrolled asthma, defined as an ACQ-6 score <1.50 or ACT or CACT score<20, greater airflow limitation evidenced by lower FEV_1/FVC and FEF_{25-75}% predicted values, and a more prevalent exacerbation occurrence during the observation interval (Table IV). The associations between high ECP concentrations and exacerbation occurrence, but not high ECP concentrations and lung function or asthma control, persisted after adjustment for sex, race, household educational attainment, and tobacco smoke exposure (Table E2, available in this article's Online Repository at www.jaci-inpractice.org). However, the sensitivity of ECP for detecting exacerbation occurrence was modest and was not markedly different from that of blood eosinophil counts or exhaled nitric oxide concentrations measured at the baseline visit (Figure 4). Inspection of the receiver operating characteristic curve revealed that lower ECP cut-points provide the best sensitivity, with modest specificity (Table E3, available in this article's Online Repository at www.jaci-inpractice.org).

DISCUSSION

This study addressed whether plasma ECP concentrations could detect type 2 inflammation and discriminate clinical outcomes in children with atopic asthma. In this cohort, higher ECP concentrations were associated with higher blood eosinophil counts, aeroallergen sensitization, IgE and exhaled nitric oxide concentrations and were further associated with poorer asthma control, greater airflow limitation, and more exacerbations both at baseline and at 12 months of follow-up. Children with higher ECP concentrations also had greater improvement in asthma control with the initiation of systemic corticosteroids. These observations suggest that plasma ECP concentrations may be a useful marker of type 2 inflammation in children and may help identify those children at highest risk for recurrent exacerbations who could benefit from corticosteroid treatment. The fact that ECP concentrations remained elevated after intramuscular triamcinolone, unlike blood eosinophils or exhaled nitric oxide,

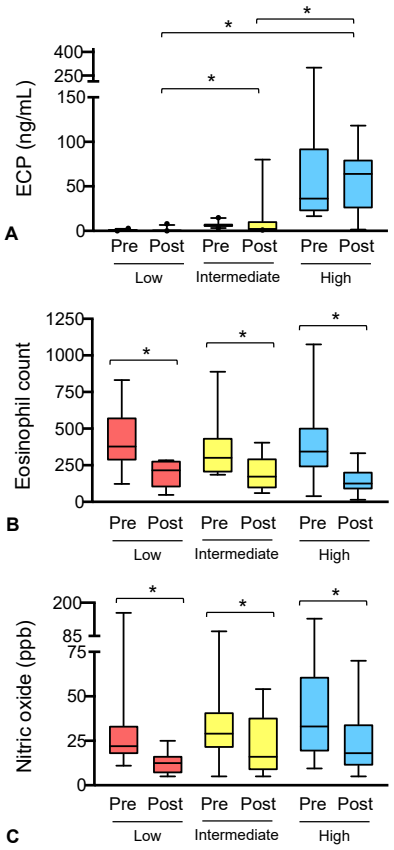


FIGURE 3. (**A**) Eosinophil cationic protein (ECP) concentration, (**B**) blood eosinophil counts, and (**C**) exhaled nitric oxide concentrations in Low ECP (red), Intermediate ECP (yellow), and High ECP (blue) groups, before and after triamcinolone administration. $*P < .05$.

also suggests that ECP may be less subject to treatment confounding. However, we also show that, similar to blood eosinophil counts or exhaled nitric oxide, the sensitivity of ECP for predicting exacerbation is relatively poor. Additional biomarkers of type 2 inflammation, or combinations of existing biomarkers, may ultimately be needed in routine clinical practice.

The results of our study are similar to others that have shown associations between ECP and features of type 2 inflammation, namely blood eosinophils,[29] allergen sensitization[30,31] and exhaled nitric oxide,[29] in children with asthma. Surprisingly, we did not observe correlations between ECP and the type 2 cytokines IL-4, IL-5, IL-13, or CCL-11/eotaxin, but these cytokines have not been consistently detected in children with asthma, including those with severe refractory asthma who also have airway tissue eosinophilia.[32,33] There is also no gold standard for type 2 inflammation for the purpose of comparison. The original studies of type 2 "high" asthma relied on epithelial gene expression signatures to define immune phenotypes,[34,35] but bronchoscopy is not clinically indicated in most patients with asthma and these epithelial-based definitions are therefore not practical in pediatric outpatient settings. Although studies of exhaled nitric oxide or blood eosinophils have shown promise, cut-points for these markers also remain debatable[8] because these markers may have little concordance in the same patient[36,37] and may be subject to significant variability over time and in response to asthma treatments.[38]

Nonetheless, our study results do have biologic plausibility. Multiple *in vitro* studies have shown that high levels of ECP are cytoxic and promote fibroblast migration and the interaction between mast cells and eosinophils in some tissue.[12] Although the functional impact of physiologic concentrations of ECP in humans is still unclear, others have similarly shown that children with higher ECP concentrations have poorer symptom control[39] and more airflow obstruction[29,40] with greater bronchodilator responsiveness[41] in small, cross-sectional studies. The present study advances these observations to a large cohort of racially-diverse children who were followed longitudinally for 12 months and demonstrates that the clinical features of type 2 "high" asthma that are discriminated by high plasma ECP concentrations are relatively stable over time. The present study is also the first to demonstrate that ECP concentrations can predict future exacerbations and systemic corticosteroid treatment responsiveness. The fact that ECP concentrations were highly correlated with total IgE levels is also of interest, because previous studies have shown that blood eosinophils may predict the clinical responses to anti-IgE treatment initiation.[42-44] Whether ECP would be similarly useful in prediction of anti-IgE therapeutic responses or responses to other biologics directed at type 2 inflammation is unclear and warrants further study.

Strengths of the present study are the large and highly characterized sample that permitted adjustment for key covariates (sex, race, household educational attainment, and tobacco smoke exposure) that are known to be associated with asthma outcomes.[45] The large proportion of enrolled children with an asthma exacerbation treated with systemic corticosteroids in the previous year also ensured a study population with sufficient disease burden at greatest risk for poorer outcomes. We also elected to measure ECP in the plasma, unlike the majority of other pediatric asthma studies,[5,14,29-31,39-41,46-48] because the anticoagulant EDTA attenuates eosinophil ECP release and provides a better indication of the *in situ* ECP concentration.[10,12] Serum ECP levels are also highly dependent on sample handling and increase with rising sample temperatures during the clotting process.[49] Serum ECP levels are therefore considerably higher (ie, approximately 2-fold) than plasma ECP levels.[49] Despite the limited number of studies of plasma ECP in children with asthma, one study of plasma ECP concentrations in hospitalized children with acute asthma observed a median ECP concentration of 2.8 ng/mL (with a range up to 15 ng/mL), which decreased after systemic corticosteroid administration.[50] Two other studies of nonatopic healthy adults noted that mean plasma ECP concentrations were approximately 3.0 ng/mL,[49,51] which is similar to the cut-point of 3.12 ng/mL, which was used for the lowest ECP tertile in the present study.

However, this study does have limitations. First, this study was limited to children with atopic asthma. The majority of these children had allergic rhinitis and eczema, which also may influence ECP measurements. Therefore, it is unclear whether ECP would perform similarly as a predictor of corticosteroid responsiveness and exacerbations in more heterogeneous populations of children with diverse patterns of inflammation. We were also unable to measure ECP in the airways, and it is possible that systemic ECP may not adequately reflect airway tissue eosinophilia, which can persist for 7 to 14 days after eosinophils

TABLE III. Asthma outcomes after intramuscular triamcinolone administration, by baseline eosinophil cationic protein (ECP) tertile

	Low ECP (<3.12 ng/mL)	Intermediate ECP (3.12-16.22 ng/mL)	High ECP (≥16.23 ng/mL)
ACQ-6 score	0.55 ± 0.12	0.95 ± 0.22	0.96 ± 0.14*
Mean difference from baseline†	0.16 ± 0.14	0.29 ± 0.16	0.36 ± 0.14*
PAQLQ score	6.24 ± 0.14	5.77 ± 0.22	5.79 ± 0.16
Mean difference from baseline	−0.60 ± 0.16	−0.48 ± 0.18	−0.48 ± 0.12
FEV_1 (% predicted)	100.1 ± 2.7	93.6 ± 3.5	88.4 ± 2.9*
Mean difference from baseline	−0.97 ± 1.78	0.61 ± 2.92	−2.66 ± 2.46
FEV_1/FVC (% predicted)	93.9 ± 1.6	89.7 ± 2.4	87.4 ± 1.8*
Mean difference from baseline	0.79 ± 1.63	0.25 ± 1.72	2.10 ± 1.41

Data represent the mean ± standard error of the mean.
ACQ, Asthma Control Questionnaire; *FEV_1*, forced expiratory volume in 1 second; *FVC*, forced vital capacity; *PAQLQ*, Pediatric Asthma Quality of Life Questionnaire.
*$P < .05$ vs Low ECP.
†Pre-triamcinolone value minus post-triamcinolone value.

TABLE IV. Asthma features at 12 months, by baseline eosinophil cationic protein (ECP) tertile

	Low ECP (<3.12 ng/mL)	Intermediate ECP (3.12-16.22 ng/mL)	High ECP (≥16.23 ng/mL)
Type 2 inflammatory markers			
ECP (ng/mL)	1.28 ± 0.44	19.50 ± 6.07*	62.29 ± 6.12*
Blood eosinophils (cells/μL)	244.6 ± 27.2	353.1 ± 43.9*	271.1 ± 31.4
Blood eosinophils (%)	3.60 ± 0.45	6.10 ± 0.84*	4.73 ± 0.47*
Exhaled nitric oxide (ppb)	25.8 ± 5.2	40.7 ± 5.0*	36.0 ± 28.4*
Asthma control†			
ACQ-6 score	0.63 ± 0.13	1.18 ± 0.14	1.00 ± 0.14
ACT or CACT score	22.1 ± 0.5	19.1 ± 0.8	20.2 ± 0.7
Uncontrolled asthma	12 (27.3)	21 (51.2)*	23 (46.0)*
PAQLQ score‡	6.01 ± 0.14	5.83 ± 0.17	5.83 ± 0.20
Lung function‡			
FVC (% predicted)	101.9 ± 2.3	103.5 ± 2.8	101.9 ± 1.4
FEV_1 (% predicted)	94.4 ± 2.2	92.7 ± 3.1	88.7 ± 2.3
FEV_1/FVC (% predicted)	92.5 ± 1.8	89.3 ± 2.1	87.0 ± 1.6*
FEF_{25-75} (% predicted)	80.2 ± 4.8	73.4 ± 4.8	66.9 ± 3.9*
Exacerbation history, past 12 mo§			
Any systemic corticosteroid burst	20 (28.2)	23 (36.5)	35 (50.0)*
Any emergency department visit	19 (26.8)	14 (22.2)	31 (44.3)*
Any hospitalization	3 (4.2)	3 (4.8)	12 (17.2)*

Data represent the mean ± standard error of the mean or the number of participants (%).
ACQ, Asthma Control Questionnaire; *ACT*, Asthma Control Test; *CACT*, Childhood Asthma Control Test; *FEF_{25-75}*, forced expiratory flow at 25%-75% of vital capacity; *FEV_1*, forced expiratory volume in 1 second; *FVC*, forced vital capacity; *PAQLQ*, Pediatric Asthma Quality of Life Questionnaire.
*$P < .05$ vs low ECP.
†Low ECP, n = 44; Intermediate ECP, n = 41; High ECP, n = 50.
‡Low ECP, n = 45; Intermediate ECP, n = 39; High ECP, n = 47.
§Low ECP, n = 71; Intermediate ECP, n = 63, High ECP, n = 70.

exit the bloodstream.[9] Although a previous study in adults noted correlations between systemic ECP and bronchoalveolar lavage and airway tissue eosinophils, the associations were of moderate nature (bronchoalveolar lavage eosinophils, $r = 0.457$, $R^2 = 0.209$; tissue eosinophils, $r = 0.556$, $R^2 = 0.309$).[52] Airway biomarker measurement is also invasive and difficult to perform in general populations of children. It is also recognized that our selected outcomes of asthma control and exacerbations are multifactorial and could be influenced by unmeasured sources of confounding including adherence to asthma medications, which was not adequately assessed. It is therefore unclear whether the outcomes at 12 months or the triamcinolone response outcomes observed in the present study would have differed with supervised inhaled corticosteroid treatment. Indeed, other highly controlled clinical trials of preschool and school-age children have shown that systemic ECP measures predict symptom and lung function improvement with inhaled corticosteroid initiation.[5,14] However, it is also recognized that there are type 2 "high" refractory asthma phenotypes in children that are poorly responsive to inhaled corticosteroids, which this study did not adequately assess.[1]

Other important limitations of the study include the measurement of ECP and type 2 biomarkers at a limited number of time points, which may not be optimal for assessment of type 2 inflammation. For example, a previous study in adults found that "same day" measurements of type 2 biomarkers (including blood eosinophils) identified only half of the eosinophilic group.[37] However, there is no gold standard measure of type 2 inflammation, so it is unclear whether more frequent measurement of ECP would increase the sensitivity of the biomarker. Indeed,

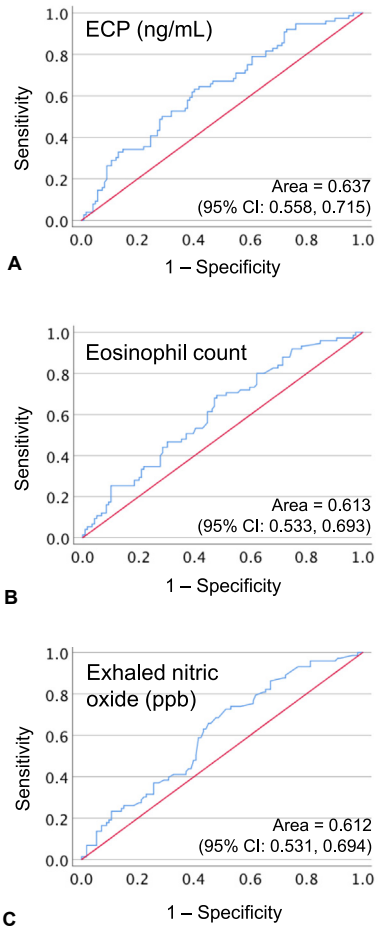


FIGURE 4. Sensitivity and specificity of baseline (**A**) eosinophil cationic protein (ECP) concentrations, (**B**) blood eosinophil counts, and (**C**) exhaled nitric oxide concentrations for detection of exacerbation occurrence by 12 months. *CI*, Confidence interval.

other studies have shown that the sensitivity of blood eosinophils is only marginally increased with multiple measures.[53] In addition, although we collected blood samples within a 5-hour time window, eosinophils are subject to some diurnal variability.[54] It is therefore likely that ECP may have similar variation.

In summary, we demonstrate that plasma ECP concentrations may be a useful biomarker of type 2 inflammation, corticosteroid treatment response, and exacerbation outcomes in children with asthma. Although independent replication and validation of our results is essential, these findings offer additional insight on the importance and measurement of eosinophil activation in children with asthma. Whether children with elevated plasma ECP concentrations also respond differently to other management strategies for type 2 inflammation, such as biologics, is unclear and warrants further evaluation.

REFERENCES

1. Pijnenburg MW, Fleming L. Advances in understanding and reducing the burden of severe asthma in children. Lancet Respir Med 2020;8:1032-44.
2. Grunwell JR, Gillespie S, Morris CR, Fitzpatrick AM. Latent class analysis of school-age children at risk for asthma exacerbation. J Allergy Clin Immunol Pract 2020;8:2275-2284.e2.
3. Fitzpatrick AM, Bacharier LB, Guilbert TW, Jackson DJ, Szefler SJ, Beigelman A, et al. Phenotypes of recurrent wheezing in preschool children: identification by latent class analysis and utility in prediction of future exacerbation. J Allergy Clin Immunol Pract 2019;7:915-924.e7.
4. Fitzpatrick AM, Bacharier LB, Jackson DJ, Szefler SJ, Beigelman A, Cabana M, et al. Heterogeneity of mild to moderate persistent asthma in children: confirmation by latent class analysis and association with 1-year outcomes. J Allergy Clin Immunol Pract 2020;8:2617-2627.e4.
5. Fitzpatrick AM, Jackson DJ, Mauger DT, Boehmer SJ, Phipatanakul W, Sheehan WJ, et al. Individualized therapy for persistent asthma in young children. J Allergy Clin Immunol 2016;138:1608-1618.e12.
6. Busby J, Khoo E, Pfeffer PE, Mansur AH, Heaney LG. The effects of oral corticosteroids on lung function, type-2 biomarkers and patient-reported outcomes in stable asthma: a systematic review and meta-analysis. Respir Med 2020;173:106156.
7. Coverstone AM, Seibold MA, Peters MC. Diagnosis and management of T2-high asthma. J Allergy Clin Immunol Pract 2020;8:442-50.
8. Shah SP, Grunwell J, Shih J, Stephenson S, Fitzpatrick AM. Exploring the utility of noninvasive type 2 inflammatory markers for prediction of severe asthma exacerbations in children and adolescents. J Allergy Clin Immunol Pract 2019;7:2624-2633.e2.
9. Aoki A, Hirahara K, Kiuchi M, Nakayama T. Eosinophils: cells known for over 140 years with broad and new functions. Allergol Int 2021;70:3-8.
10. Malm-Erjefalt M, Greiff L, Ankerst J, Andersson M, Wallengren J, Cardell LO, et al. Circulating eosinophils in asthma, allergic rhinitis, and atopic dermatitis lack morphological signs of degranulation. Clin Exp Allergy 2005;35:1334-40.
11. Peterson CG, Enander I, Nystrand J, Anderson AS, Nilsson L, Venge P. Radioimmunoassay of human eosinophil cationic protein (ECP) by an improved method. Establishment of normal levels in serum and turnover in vivo. Clin Exp Allergy 1991;21:561-7.
12. Bystrom J, Amin K, Bishop-Bailey D. Analysing the eosinophil cationic protein—a clue to the function of the eosinophil granulocyte. Respir Res 2011;12:10.
13. Koh GC, Shek LP, Goh DY, Van Bever H, Koh DS. Eosinophil cationic protein: is it useful in asthma? A systematic review. Respir Med 2007;101:696-705.
14. Szefler SJ, Phillips BR, Martinez FD, Chinchilli VM, Lemanske RF, Strunk RC, et al. Characterization of within-subject responses to fluticasone and montelukast in childhood asthma. J Allergy Clin Immunol 2005;115:233-42.
15. Kato M, Yamada Y, Maruyama K, Hayashi Y. Differential effects of corticosteroids on serum eosinophil cationic protein and cytokine production in rhinovirus- and respiratory syncytial virus-induced acute exacerbation of childhood asthma. Int Arch Allergy Immunol 2011;155(Suppl 1):77-84.
16. Stelmach I, Jerzynska J, Kuna P. Markers of allergic inflammation in peripheral blood of children with asthma after treatment with inhaled triamcinolone acetonide. Ann Allergy Asthma Immunol 2001;87:319-26.
17. Lonnkvist K, Hellman C, Lundahl J, Hallden G, Hedlin G. Eosinophil markers in blood, serum, and urine for monitoring the clinical course in childhood asthma: impact of budesonide treatment and withdrawal. J Allergy Clin Immunol 2001;107:812-7.
18. Prehn A, Seger RA, Torresani T, Molinari L, Sennhauser FH. Evaluation of a clinical algorithm involving serum eosinophil cationic protein for guiding the anti-inflammatory treatment of bronchial asthma in childhood. Pediatr Allergy Immunol 2000;11:87-94.
19. Fuhlbrigge A, Peden D, Apter AJ, Boushey HA, Camargo CA Jr, Gern J, et al. Asthma outcomes: exacerbations. J Allergy Clin Immunol 2012;129:S34-48.
20. Juniper EF, Svensson K, Mork AC, Stahl E. Measurement properties and interpretation of three shortened versions of the Asthma Control Questionnaire. Respir Med 2005;99:553-8.
21. Juniper EF, Gruffydd-Jones K, Ward S, Svensson K. Asthma Control Questionnaire in children: validation, measurement properties, interpretation. Eur Respir J 2010;36:1410-6.
22. Nathan RA, Sorkness CA, Kosinski M, Schatz M, Li JT, Marcus P, et al. Development of the Asthma Control Test: a survey for assessing asthma control. J Allergy Clin Immunol 2004;113:59-65.
23. Liu AH, Zeiger R, Sorkness C, Mahr T, Ostrom N, Burgess S, et al. Development and cross-sectional validation of the Childhood Asthma Control Test. J Allergy Clin Immunol 2007;119:817-25.
24. Juniper EF, Guyatt GH, Feeny DH, Ferrie PJ, Griffith LE, Townsend M. Measuring quality of life in children with asthma. Qual Life Res 1996;5:35-46.
25. Juniper EF, Guyatt GH, Feeny DH, Griffith LE, Ferrie PJ. Minimum skills required by children to complete health-related quality of life instruments for asthma: comparison of measurement properties. Eur Respir J 1997;10:2285-94.
26. American Thoracic Society, European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement

of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med 2005;171:912-30.
27. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. Eur Respir J 2005;26:319-38.
28. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J 2012;40:1324-43.
29. Strunk RC, Szefler SJ, Phillips BR, Zeiger RS, Chinchilli VM, Larsen G, et al. Relationship of exhaled nitric oxide to clinical and inflammatory markers of persistent asthma in children. J Allergy Clin Immunol 2003;112:883-92.
30. Patelis A, Alving K, Middelveld R, James A, Ono J, Ohta S, et al. IgE sensitization to food allergens and airborne allergens in relation to biomarkers of type 2 inflammation in asthma. Clin Exp Allergy 2018;48:1147-54.
31. Lee YJ, Kim KW, Choi BS, Sohn MH, Kim KE. Clinical characteristics of eosinophilic and noneosinophilic asthma in children. Acta Paediatr 2013;102:53-7.
32. Bossley CJ, Fleming L, Gupta A, Regamey N, Frith J, Oates T, et al. Pediatric severe asthma is characterized by eosinophilia and remodeling without T(H)2 cytokines. J Allergy Clin Immunol 2012;129:974-982.e13.
33. de Blic J, Tillie-Leblond I, Tonnel AB, Jaubert F, Scheinmann P, Gosset P. Difficult asthma in children: an analysis of airway inflammation. J Allergy Clin Immunol 2004;113:94-100.
34. Hekking PP, Loza MJ, Pavlidis S, De Meulder B, Lefaudeux D, Baribaud F, et al. Transcriptomic gene signatures associated with persistent airflow limitation in patients with severe asthma. Eur Respir J 2017;50:1602298.
35. Kuo CS, Pavlidis S, Loza M, Baribaud F, Rowe A, Pandis I, et al. T-helper cell type 2 (Th2) and non-Th2 molecular phenotypes of asthma using sputum transcriptomics in U-BIOPRED. Eur Respir J 2017;49:1602135.
36. Hastie AT, Moore WC, Li H, Rector BM, Ortega VE, Pascual RM, et al. Biomarker surrogates do not accurately predict sputum eosinophil and neutrophil percentages in asthmatic subjects. J Allergy Clin Immunol 2013;132:72-80.
37. Haughney J, Morice A, Blyth KG, Lee AJ, Coutts A, McKnight E, et al. A retrospective cohort study in severe asthma describing commonly measured biomarkers: eosinophil count and IgE levels. Respir Med 2018;134:117-23.
38. Mathur SK, Fichtinger PS, Evans MD, Schwantes EA, Jarjour NN. Variability of blood eosinophil count as an asthma biomarker. Ann Allergy Asthma Immunol 2016;117:551-3.
39. Kim CK, Callaway Z, Fletcher R, Koh YY. Eosinophil-derived neurotoxin in childhood asthma: correlation with disease severity. J Asthma 2010;47:568-73.
40. Lee YJ, Lee HH, Choi BS, Jee HM, Kim KW, Sohn MH, et al. Association between eosinophilic airway inflammation and persistent airflow limitation. J Asthma 2013;50:342-6.
41. Yoo Y, Seo SC, Kim YI, Chung BH, Song DJ, Choung JT. Bronchodilator responses after methacholine and adenosine 5'-monophosphate (AMP) challenges in children with asthma: their relationships with eosinophil markers. J Asthma 2012;49:717-23.
42. Casale TB, Luskin AT, Busse W, Zeiger RS, Trzaskoma B, Yang M, et al. Omalizumab effectiveness by biomarker status in patients with asthma: evidence from PROSPERO, a prospective real-world study. J Allergy Clin Immunol Pract 2019;7:156-164.e1.
43. Sorkness CA, Wildfire JJ, Calatroni A, Mitchell HE, Busse WW, O'Connor GT, et al. Reassessment of omalizumab-dosing strategies and pharmacodynamics in inner-city children and adolescents. J Allergy Clin Immunol Pract 2013;1:163-71.
44. Humbert M, Taille C, Mala L, Le Gros V, Just J, Molimard M, et al. Omalizumab effectiveness in patients with severe allergic asthma according to blood eosinophil count: the STELLAIR study. Eur Respir J 2018;51:1702523.
45. Pongracic JA, Krouse RZ, Babineau DC, Zoratti EM, Cohen RT, Wood RA, et al. Distinguishing characteristics of difficult-to-control asthma in inner-city children and adolescents. J Allergy Clin Immunol 2016;138:1030-41.
46. Lonnkvist K, Anderson M, Hedlin G, Svartengren M. Exhaled NO and eosinophil markers in blood, nasal lavage and sputum in children with asthma after withdrawal of budesonide. Pediatr Allergy Immunol 2004;15:351-8.
47. Mogensen I, Alving K, Bjerg A, Borres MP, Hedlin G, Sommar J, et al. Simultaneously elevated exhaled nitric oxide and serum-eosinophil cationic protein relate to recent asthma events in asthmatics in a cross-sectional population-based study. Clin Exp Allergy 2016;46:1540-8.
48. Koh YY, Kang H, Kim CK. Ratio of serum eosinophil cationic protein/blood eosinophil counts in children with asthma: comparison between acute exacerbation and clinical remission. Allergy Asthma Proc 2003;24:269-74.
49. Bjork A, Venge P, Peterson CG. Measurements of ECP in serum and the impact of plasma coagulation. Allergy 2000;55:442-8.
50. Ip WK, Wong CK, Leung TF, Lam CW. Plasma concentrations of soluble CTLA-4, CD28, CD80 and CD86 costimulatory molecules reflect disease severity of acute asthma in children. Pediatr Pulmonol 2006;41:674-82.
51. Park YJ, Oh EJ, Park JW, Kim M, Han K. Plasma eosinophil cationic protein, interleukin-5, and ECP/Eo count ratio in patients with various eosinophilic diseases. Ann Clin Lab Sci 2006;36:262-6.
52. Niimi A, Amitani R, Suzuki K, Tanaka E, Murayama T, Kuze F. Serum eosinophil cationic protein as a marker of eosinophilic inflammation in asthma. Clin Exp Allergy 1998;28:233-40.
53. Katz LE, Gleich GJ, Hartley BF, Yancey SW, Ortega HG. Blood eosinophil count is a useful biomarker to identify patients with severe eosinophilic asthma. Ann Am Thorac Soc 2014;11:531-6.
54. Durrington HJ, Gioan-Tavernier GO, Maidstone RJ, Krakowiak K, Loudon ASI, Blaikley JF, et al. Time of day affects eosinophil biomarkers in asthma: implications for diagnosis and treatment. Am J Respir Crit Care Med 2018;198:1578-81.

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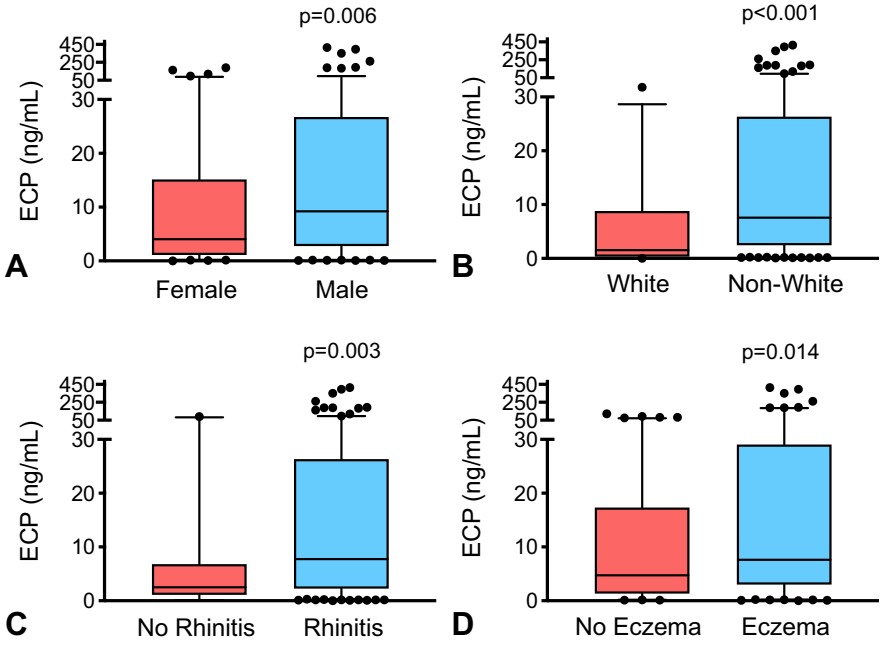

FIGURE E1. Associations between eosinophil cationic protein (ECP) and (**A**) sex, (**B**) race, (**C**) allergic rhinitis, and (**D**) eczema. Boxplot whiskers represent 5th and 95th percentiles. Dots represent individual outliers (participants).

TABLE E1. Association between demographic variables and eosinophil cationic protein (ECP) concentration

Model	Predictor	β	SE	*P* value
Model 1 (unadjusted)	Sex (female = 0, male = 1)	0.645	0.232	.006
	Race (white = 0, non-white = 1)	1.585	0.353	<.001
	Allergic rhinitis (no = 0, yes = 1)	0.997	0.327	.003
	Eczema (no = 0, yes = 1)	0.570	0.230	.014
Model 2 (adjusted)*	Sex (female = 0, male = 1)	0.569	0.136	.049
	Race (white = 0, non-white = 1)	1.394	0.346	<.001
	Allergic rhinitis (no = 0, yes = 1)	0.812	0.321	.012
	Eczema (no = 0, yes = 1)	0.516	0.223	.022
Model 3 (adjusted)†	Sex (female = 0, male = 1)	0.444	0.227	.051
	Race (white = 0, non-white = 1)	1.344	0.342	<.001
	Allergic rhinitis (no = 0, yes = 1)	0.718	0.318	.025
	Eczema (no = 0, yes = 1)	0.543	0.220	.014
Model 4 (adjusted)‡	Sex (female = 0, male = 1)	0.492	0.224	.029
	Race (white = 0, non-white = 1)	1.303	0.343	<.001
	Allergic rhinitis (no = 0, yes = 1)	0.696	0.333	.038
	Eczema (no = 0, yes = 1)	0.457	0.227	.045

ECP was modeled as a log concentration.
SE, Standard error.
*Adjusted for blood eosinophil count (log value).
†Adjusted for blood eosinophil count (log value), inhaled corticosteroid dose (none = 0; low-medium = 1; high = 2).
‡Adjusted for blood eosinophil count (log value), inhaled corticosteroid dose (none = 0; low-medium = 1; high = 2); uncontrolled asthma (0 = no, 1 = yes).

TABLE E2. Association between ECP tertile at the baseline visit and asthma outcomes assessed at 12 months

		Unadjusted model				Adjusted model*			
Outcome at 12 months	**Variable**	**β**	**SE**	**OR (95% CI)**	***P* value**	**β**	**SE**	**OR (95% CI)**	***P* value**
Uncontrolled asthma (yes/no)	Intermediate vs Low ECP	1.04	0.48	2.82 (1.10, 7.19)	.030	0.96	0.51	2.62 (0.95, 7.22)	.062
	High vs Low ECP	0.91	0.45	2.49 (1.03, 6.06)	.044	0.74	0.51	2.11 (0.77, 5.74)	.146
Exacerbation occurrence (yes/no)	Intermediate vs Low ECP	0.38	0.37	1.47 (0.71, 3.04)	.303	0.26	0.39	1.29 (0.60, 2.80)	.512
	High vs Low ECP	0.94	0.36	2.55 (1.27, 5.12)	.009	0.79	0.39	2.22 (1.02, 4.81)	.042
FEV_1/FVC (% predicted value)	ECP tertile	−2.69	1.23	—	.032	−2.30	1.32	—	.086
FEF_{25-75} (% predicted value)	ECP tertile	−6.62	3.04	—	.031	−4.96	3.25	—	.130

CI, Confidence interval; *ECP*, eosinophil cationic protein; FEF_{25-75}, forced expiratory flow at 25%-75% of vital capacity; FEV_1, forced expiratory volume in 1 second; *FVC*, forced vital capacity; *OR*, odds ratio; *SE*, standard error.
*Adjusted for sex, race, tobacco smoke exposure, and household educational attainment.

TABLE E3. Sensitivity and specificity of selected cut-points for plasma eosinophil cationic protein (ECP) concentrations for the detection of exacerbation at 12 months

Cut-point (ng/mL ECP)	**Sensitivity**	**1 − Specificity**
2.0	0.816	0.664
2.5	0.789	0.632
3.0	0.750	0.598
3.5	0.711	0.574
4.0	0.697	0.549
4.5	0.671	0.538
5.0	0.671	0.465
5.5	0.658	0.457