

Elevated Eosinophil Protein X in Urine from Healthy Neonates Precedes Development of Atopy in the First 6 Years of Life

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Rationale: Biomarkers predicting development of atopic disease are needed for targeted preventive measures and to study if disease pathology may be active before onset of symptoms.

Objectives: To investigate whether eosinophil protein X, leukotriene-C₄/D₄/E₄, and 11 β -prostaglandin (PG) F_{2 α} (PGD₂ metabolite) assessed in urine from healthy at-risk neonates precede development of atopic disease during the first 6 years of life.

Methods: We measured eosinophil protein X (n = 369), leukotriene-C₄/D₄/E₄ (n = 367), and 11 β -PGF_{2 α} (n = 366) in urine from 1-month-old children participating in the Copenhagen Prospective Studies on Asthma in Childhood birth cohort. Clinical data on development of allergic sensitization, allergic rhinitis, nasal eosinophilia, blood eosinophilia, eczema, troublesome lung symptoms (significant cough or wheeze or dyspnea), and asthma were collected prospectively until age 6 years. Associations between urinary biomarkers and development of atopic traits were investigated using general estimating equations, logistic regression, and Cox regression.

Measurements and Main Results: Eosinophil protein X in the urine of the asymptomatic 1-month-old neonates was significantly associated with development of allergic sensitization (odds ratio, 1.49; 95% confidence interval [CI], 1.08–1.89), nasal eosinophilia (odds ratio, 3.2; 95% CI, 1.2–8.8), and eczema (hazard ratio, 1.4; 95% CI, 1.0–2.0), but not with allergic rhinitis, asthma, or blood eosinophilia. Neither leukotriene-C₄/D₄/E₄ nor 11 β -PGF_{2 α} was associated with any of the atopic phenotypes.

Conclusions: Eosinophil protein X in urine from asymptomatic neonates is a biomarker significantly associated with later development of allergic sensitization, nasal eosinophilia, and eczema during the first 6 years of life. These findings suggest activation of eosinophil granulocytes early in life before development of atopy-related symptoms.

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Urinary eosinophil protein X is elevated in subjects with present allergic sensitization and has been associated with severity of eczema and acute and chronic childhood asthma.

What This Study Adds to the Field

Elevated level of urinary eosinophil protein X in symptom-free high-risk neonates precedes development of allergic sensitization, nasal eosinophilia, and eczema during pre-school age suggesting eosinophilic activation before the development of atopy-related illnesses.

Keywords: atopy; blood eosinophils; eczema; neonates; urinary eosinophil protein X

Identification of children at high risk of developing atopic disorders is a major research challenge. Biomarkers predicting development of atopic disease before the onset of symptoms are needed for targeted prevention and individualized intervention and treatment. Furthermore, it is an important research question if the disease process may be active before development of symptoms as we previously reported on increased fraction of exhaled nitric oxide in neonates later developing early wheeze (1) and pathogenic bacterial colonization of the airways in neonates later developing asthma (2).

Urinary eosinophil protein X (u-EPX) released from activated eosinophil granulocytes is seen in association with current allergic sensitization (3) and has been associated with severity of eczema (4) and acute and chronic childhood asthma (3, 5, 6). Urinary leukotriene (LT) C₄/D₄/E₄ (u-LT_{C₄/D₄/E₄}), a measure of total body cysteinyl LT production, is elevated in subjects with symptomatic allergic rhinitis (7) and in preschoolers with acute wheeze illnesses (8, 9). Prostaglandin (PG) D₂, the major cyclooxygenase product from activated mast cells, has been associated with allergen-induced asthma illustrated by elevated urinary level of 11 β -PGF_{2 α} (u-11 β -PGF_{2 α}), which is a major stable PGD₂ metabolite (10). Thus, u-EPX, u-LT_{C₄/D₄/E₄}, and u-11 β -PGF_{2 α} are all well-known markers of established atopic inflammation, but it is unknown whether levels are elevated very early in life preceding symptom debut.

We measured u-EPX, u-LT_{C₄/D₄/E₄}, and u-11 β -PGF_{2 α} in healthy high-risk 1-month-old asymptomatic neonates to investigate whether elevated level of these biomarkers precede subsequent development of atopic disease during the first 6 years of life. We used the comprehensive longitudinal clinical data on troublesome lung symptoms, asthma, eczema, rhinitis, allergic sensitization, blood, and nasal eosinophils in the Copenhagen Prospective Study on Asthma in Childhood (COPSAC) birth cohort of high-risk babies.

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We hypothesized that elevated levels of u-EPX, u-LT_{C4/D4/E4}, and u-11 β -PGF_{2 α} very early in life reflects presymptomatic disease activity preceding later development of atopic disease.

METHODS

Study Design

The COPSAC birth cohort is a prospective clinical study of 411 children born to mothers with asthma previously described in detail (2, 11, 12). The symptom-free children were enrolled at 1 month of age and subsequently attended the clinical research unit for scheduled clinical investigations at 6-monthly intervals and immediately on onset of any respiratory or skin-related symptom. Key exclusion criteria were gestational age less than 36 weeks, severe congenital abnormality, neonatal mechanical ventilation and symptoms of lower airway infection before 1 month of age.

The study was conducted in accordance with the Declaration of Helsinki and was approved by The Copenhagen Ethics Committee (KF 01-289/96) and The Danish Data Protection Agency (2008-41-1754) (11). Informed consent was obtained from both parents before enrollment.

Urine Inflammatory Markers

Urine was collected during visits to the COPSAC research unit at ages 1 and 6 months into a sterile plastic bag adherent to the skin. The urine samples were immediately transferred to 3.6-ml Nunc tubes, and the aliquots were stored without addition of any preservatives at -80°C until analysis.

After thawing, levels of EPX, LT_{C4/D4/E4}, and 11 β -PGF_{2 α} were measured in the urine samples. u-EPX concentration was determined by a double-antibody immunoassay (radioimmunoassay; Pharmacia Upjohn, AB, Uppsala, Sweden) in both the 1- and 6-month samples. The combined quantitative value of u-LT_{C4/D4/E4} levels was measured by ELISA test kits (Neogen Corporation, Lexington, KY). Urinary level of 11 β -PGF_{2 α} was also assessed by ELISA test kits (Neogen Corporation).

To standardize for variations in renal excretion, levels of all urinary markers were adjusted for creatinine excretion (13). Urinary creatinine levels were measured by buffered kinetic Jaffé reaction (Cobas Integra 700 analyzer; Roche Diagnostics, Hvidovre, Denmark). All urine laboratory analyses were performed at Phadia ApS, Allerød, Denmark.

Atopic Intermediary End Points

Allergic sensitization was determined at ages 6 and 18 months and 4 and 6 years by measurement of serum-specific IgE against 15 common inhalant and food allergens (cat, dog, horse, birch, timothy grass, mugwort, house dust mites, molds, hen's egg, cow's milk, fish, wheat, peanut, soybean, and shrimp) by ImmunoCAP assay (Pharmacia Diagnostics AB, Uppsala, Sweden). Sensitization was defined as specific-IgE greater than or equal to 0.35 kU/L (14, 15) for any of the tested allergens and was analyzed as a dichotomized measurement.

In a secondary analysis we stratified presence of allergic sensitization as (1) food sensitization, being any sensitization for hen's egg, cow's milk, fish, wheat, peanut, soybean, or shrimp; (2) sensitization for inhaled allergens, being any sensitization for cat, dog, horse, birch, timothy grass, mugwort, house dust mites, or molds; (3) sensitization for outdoor aeroallergens, being any sensitization for birch, timothy grass, or mugwort; and (4) sensitization for indoor aeroallergens, being any sensitization for cat, dog, house dust mites, or molds.

Blood eosinophil count (10^9 cells per liter) was assessed at ages 6 and 18 months and 4 and 6 years. Nasal eosinophilia was investigated by nasal scraping in the child's sixth year of life and rated according to Meltzer's semiquantitative scale (16) as previously detailed (17).

Clinical Investigator-diagnosed End Points

Allergic rhinitis was diagnosed at age 6 by the COPSAC doctors based on clinical interviews of the parents (not questionnaires) on history of symptoms in the child's sixth year of life (17-19). Rhinitis was defined as

troublesome sneezing or blocked or runny nose in the past 12 months in periods without accompanying cold or flu (20). Significance of symptoms was judged by severity, length of periods, and time of year. Allergic rhinitis was diagnosed in children with rhinitis and sensitization to aeroallergens (cat, dog, horse, birch, timothy grass, mugwort, house dust mites, or molds) clearly related to the symptomatic periods.

Troublesome lung symptoms (significant cough or wheeze or dyspnea) were recorded by the parents as a dichotomized daily score (yes or no) from birth until age 7 years (21). Recurrent troublesome lung symptoms (recently termed "multitrigger wheeze" [22]) were defined from the diaries as five episodes within 6 months, each episode lasting at least 3 consecutive days, or daily symptoms for 4 consecutive weeks (1, 23). Children meeting these criteria were prescribed a 3-month trial of budesonide, 200 μg twice a day in a pressurized metered dose inhaler with a spacer. Episodic viral troublesome lung symptoms (22) characterized children suffering discrete symptomatic episodes, with the child being well between episodes, not fulfilling the criteria for recurrent troublesome lung symptoms, and treated intermittently solely with inhaled β_2 -agonist. Early transient troublesome lung symptoms was diagnosed in children with troublesome lung symptoms at age 0-3 years but without symptoms at age 3-6 years; late-onset troublesome lung symptoms in children with troublesome lung symptoms with an age at onset at age 3-6 years; and persistent troublesome lung symptoms in children who had symptoms both at age 0-3 and 3-6 years.

Asthma at age 7 years was diagnosed by the COPSAC doctors according to international guidelines and was based on recurrent troublesome lung symptoms, symptom character, and response to trials of inhaled corticosteroids, as previously detailed (2, 12) (*see* www.copsac.com and the online supplement for further details on diagnosis of recurrent troublesome lung symptoms and asthma).

Eczema was diagnosed by using the Hanifin-Rajka criteria, as previously detailed (24, 25). Skin lesions were described at both scheduled and acute visits according to predefined morphology and localization.

Statistical Analyses

Quantitative levels of u-EPX, u-LT_{C4/D4/E4}, and u-11 β -PGF_{2 α} at age 1 month and u-EPX and blood eosinophil count at age 6 months were used as explanatory variables. Models using general estimating equations (GEE) (PROC GENMOD in SAS version 9.2; SAS Institute, Inc., Cary, NC) were applied to compute the overall odds ratio for allergic sensitization and the overall β -coefficient for blood eosinophil count using compiled data from all four time points (6 and 18 mo, 4 and 6 yr). Logistic regression was used to calculate odds ratios of the cross-sectional end points allergic rhinitis, nasal eosinophilia, asthma, and the patterns of troublesome lung symptoms from 0-6 years. Hazard ratios of recurrent troublesome lung symptoms and eczema at age 0-1 year and 0-6 years were calculated by Cox regression. Age at onset was modeled as a function of the urinary biomarkers; the children were retained in the analysis from birth until age at onset of disease, drop-out, or age at end of the observation period (i.e., 1 yr for 0-1 yr), whichever came first. Levels of urinary biomarkers and blood eosinophil count were log-transformed before analysis.

Results are reported with 95% confidence interval (CI) in brackets; a *P* value less than or equal to 0.05 was considered significant. All analyses were done in SAS version 9.2 for Windows (SAS Institute). Further details of the methods are outlined in the online supplement.

RESULTS

Baseline Characteristics

This protocol for urinary inflammatory markers was initiated after enrolling 42 of the 411 children of the cohort leaving 369 for urine analysis at age 1 month. Measurement of u-EPX was completed for all 369 children, u-LT_{C4/D4/E4} for 367, and u-11 β -PGF_{2 α} for 366, respectively. u-EPX was available for 337 children at age 6 months.

The study group consisted of 51% boys ($n = 187$). In the first year of life, 4% of the children developed recurrent troublesome lung symptoms and 27% were diagnosed with eczema. A further

17% developed recurrent troublesome lung symptoms and 15% eczema before age 6 years. Level of urinary markers, blood eosinophil count, and frequency of allergic sensitization, allergic rhinitis, nasal eosinophilia, and asthma are presented in Table 1.

There were no significant differences among the 42 subjects without urine samples and the 369 subjects in the study group with respect to sex, allergic sensitization, allergic rhinitis, nasal eosinophilia, asthma, eczema, or blood eosinophilia at age 6 years (see Table E1 in the online supplement).

u-EPX at Age 1 Month

Allergic sensitization. u-EPX at age 1 month was significantly associated with increased odds of allergic sensitization (GEE model including the four time points of 6 and 18 mo and 4 and 6 yr; odds ratio, 1.49; 95% CI, 1.08–1.89; $P = 0.02$) (Table 2), as illustrated by the plot of age-dependent odds ratios in Figure 1. Adjusting the analysis for maternal allergic sensitization assessed by specific-IgE measurements did not substantially modify the association (odds ratio, 1.45; 95% CI, 1.03–1.88; $P = 0.04$). Analyses stratified for type of sensitization: food allergens, inhaled allergens, and outdoor and indoor aeroallergens showed similar results (Table E2). Elevated u-EPX at age 1 month was associated with an increased odds of sensitization irrespective of the type of sensitization; the analysis was significant for food sensitization ($P = 0.01$), borderline significant for aeroallergen sensitization ($P = 0.06$) and sensitization to indoor inhaled allergens ($P = 0.08$), but not significant for outdoor inhaled allergens ($P = 0.38$) (Table E2).

Allergic rhinitis and nasal eosinophilia. u-EPX at age 1 month was strongly and significantly associated with nasal eosinophilia at age 6 years (odds ratio, 3.2; 95% CI, 1.2–8.8; $P = 0.02$). In addition, there was a tendency of increased odds for allergic rhinitis by age 6 years (Table 2).

Blood eosinophilia. u-EPX at 1 month was significantly associated with blood eosinophil count at age 6 years (β -coefficient, 0.19; 95% CI, 0–0.38; $P = 0.05$) (Table E3), but was not overall significantly associated with blood eosinophil count in the GEE model including all four measuring points (Table 2).

Eczema. The cumulative risk of eczema during the first 6 years of life for neonates with u-EPX level above and below the third quartile of the study group (149 $\mu\text{g/L}$) is illustrated in Figure E1, suggesting that elevated u-EPX is associated with development of eczema early in life. Accordingly, the risk of eczema during the first year of life increased significantly with increasing levels of u-EPX at age 1 month (hazard ratio, 1.4; 95% CI, 1.0–2.0; $P = 0.05$), but not thereafter (Table 3).

Troublesome lung symptoms and asthma. u-EPX was not associated with an increased risk of developing either recurrent,

episodic viral, early transient, late-onset, or persistent troublesome lung symptoms during the first 6 years of life (Tables E3 and E4), or with asthma by age 7 (Table 2, Table E4).

u-EPX at Age 6 Months

In the overall GEE model including all four time points (6 and 18 mo and 4 and 6 yr), we found an increased odds of allergic sensitization, which was borderline significant (odds ratio, 1.21; 95% CI, 0.98–1.54; $P = 0.10$). Log (u-EPX/u-creatinine) ratio at age 6 months was significantly higher in subjects with current sensitization at age 6 months (mean difference, +0.28; 95% CI, 0.00–0.56; $P = 0.05$).

u-EPX at age 6 months was significantly associated with development of allergic rhinitis at age 6 years (odds ratio, 2.3; 95% CI, 1.0–3.5; $P = 0.05$). Apart from this, the analyses of u-EPX at age 6 months showed similar association to the atopic end points as described for u-EPX at age 1 month (Tables 2 and 3). In particular, we found no association with any patterns of troublesome lung symptoms or asthma (Tables E3 and E4), and log (u-EPX/u-creatinine) ratio at age 6 months was not significantly increased in children with current troublesome lung symptoms at age 6 months (mean difference, +0.10; 95% CI, –0.25 to 0.46; $P = 0.58$).

u-LT_{C4/D4/E4} and u-11 β -PGF_{2 α} at Age 1 Month

No associations were found between these biomarkers and any of the end points studied (Tables 2 and 3).

Blood Eosinophil Count at Age 6 Months

Blood eosinophil count at age 6 months was associated with sensitization at ages 4 and 6 years, but not significantly in the overall GEE model (Table 2). Blood eosinophil count at 6 months was strongly overall associated with eosinophil counts at ages 18 months and 4 and 6 years ($\Delta\beta$, 0.41; 95% CI, 0.31–0.51; $P < 0.0001$). There was no association of blood eosinophil count at age 6 months with the development of allergic rhinitis, nasal eosinophilia, eczema, troublesome lung symptoms, or asthma (Tables 2 and 3).

DISCUSSION

Principal Findings

u-EPX in healthy 1-month-old neonates was significantly associated with development of allergic sensitization, nasal eosinophilia, and eczema during the first 6 years of life in children of the Danish COPSAC birth cohort suggesting early life eosinophilic activation before symptom debut in children developing atopy-related conditions.

TABLE 1. BASELINE CHARACTERISTICS

Variable	Age Point				
	1 Mo	6 Mo	18 Mo	4 Yr	6 Yr
u-EPX, $\mu\text{g/L}$, median (range)	83.9 (5.4–1,298)	131 (14.8–2,293)			
u-LT _{C4/D4/E4} , $\mu\text{g/L}$, median (range)	0.05 (0–19.4)				
u-11 β -PGF _{2α} , $\mu\text{g/L}$, median (range)	0.20 (0.03–1.5)				
Blood eosinophil count, $\times 10^9/\text{L}$, median (range)		0.29 (0.02–1.6)	0.21 (0.02–1.2)	0.24 (0.01–3)	0.33 (0–3.5)
Allergic sensitization, prevalence, % (n)*		7.1 (24)	11.6 (38)	26.6 (77)	36.3 (97)
Allergic rhinitis, prevalence, % (n)					9.3 (24)
Nasal eosinophilia, prevalence, % (n) [†]					6.7 (17)
Asthma, prevalence, % (n) [‡]					13.6 (41)

Definition of abbreviations: PG = prostaglandin; u-EPX = urinary eosinophil protein X; u-LT = urinary leukotriene.

* Allergic sensitization = any sensitization for cat, dog, horse, birch, timothy grass, mugwort, house dust mites, molds, hen's egg, cow's milk, fish, wheat, peanut, soybean, or shrimp.

[†] Nasal eosinophilia $\geq +1$ on Meltzer's semiquantitative scale.

[‡] Asthma at age 7 yr.

TABLE 2. ASSOCIATION AMONG URINE BIOMARKERS AT AGES 1 AND 6 MONTHS, BLOOD EOSINOPHIL COUNT AT AGE 6 MONTHS, AND DEVELOPMENT OF ALLERGIC SENSITIZATION, BLOOD EOSINOPHIL COUNT, ALLERGIC RHINITIS, NASAL EOSINOPHILIA, AND ASTHMA DURING THE FIRST 6 YEARS OF LIFE

Inflammatory Markers	Allergic Sensitization, * 0–6 Yr [†]		Blood Eosinophil Count, 0–6 Yr [†]		Allergic Rhinitis, 6 Yr		Nasal Eosinophilia, 6 Yr		Asthma, 7 Yr	
	Odds Ratio (95% CI)	P Value	β -Coefficient (95% CI)	P Value	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)	P Value	Odds Ratio (95% CI)	P Value
u-EPX, 1 mo	1.49 (1.08 to 1.89)	0.02	0.08 (−0.04 to 0.19)	0.18	1.6 (0.7 to 3.8)	0.25	3.2 (1.2 to 8.8)	0.02	1 (0.5 to 1.8)	0.99
u-LT _{C4/D4/E4} , 1 mo	1.14 (0.95 to 1.32)	0.14	0.02 (−0.04 to 0.08)	0.56	1.3 (0.8 to 2.1)	0.27	1.2 (0.8 to 1.9)	0.37	1 (0.7 to 1.4)	0.87
u-11 β -PGF _{2α} , 1 mo	1.003 (0.60 to 1.41)	0.99	−0.01 (−0.10 to 0.09)	0.90	0.6 (0.3 to 1.3)	0.20	1 (0.4 to 2.3)	0.96	0.7 (0.4 to 1.43)	0.23
u-EPX, 6 mo	1.21 (0.98 to 1.54)	0.10	0.08 (−0.02 to 0.18)	0.01	2.3 (1 to 3.5)	0.05	3 (1.4 to 5.3)	0.04	1 (0.6 to 1.8)	0.93
Blood eosinophil count, 6 mo	1.30 (0.90 to 1.69)	0.14	0.41 (0.31 to 0.51) [‡]	<0.0001	0.8 (0.4 to 1.7)	0.59	0.9 (0.4 to 2.4)	0.90	1.1 (0.6 to 2.1)	0.71

Definition of abbreviations: CI = confidence interval; PG = prostaglandin; u-EPX = urinary eosinophil protein X; u-LT = urinary leukotriene.

*Allergic sensitization = any sensitization for cat, dog, horse, birch, timothy grass, mugwort, house dust mites, molds, hen's egg, cow's milk, fish, wheat, peanut, soybean, or shrimp.

[†]Allergic sensitization and blood eosinophil count are measured at ages 6 and 18 mo and 4 and 6 yr. Models for these end points are based on general estimating equations; P values are robust score tests.

[‡]In this model the outcome was blood eosinophil count measured at ages 18 mo and 4 and 6 yr.

Strengths and Limitations of the Study

This study provides the first data of urinary inflammatory markers measured in healthy neonates before development of any atopic symptom. Previous studies have solely investigated subjects with established atopy (3–10).

The differential associations with u-EPX associating with several atopic traits during preschool age and u-LT_{C4/D4/E4} and u-11 β -PGF_{2 α} without association with any of the atopic end points make the risk of a chance finding unlikely.

It is a major strength of this study that the clinical end points were collected in a closely monitored single-center birth cohort study with comprehensive longitudinal data on development of atopic disorders. The risk of misclassification and variability in the clinical data was minimized because all diagnoses were controlled by the COPSAC doctors who acted as general practitioners for the cohort. Risk of recall bias was minimized because the families attended the research unit at half-yearly sessions and at acute symptomatic periods. Parental misinterpretation of symptoms was minimized because all mothers have a history of asthma and high prevalence of related atopic disorders, such as eczema and rhinitis, and clinical assessments were made at all visits by the COPSAC doctors.

The symptom diaries on troublesome lung symptoms (significant cough or wheeze or dyspnea) maintained by the parents day-to-day from birth until age 7 years is another significant strength of the study increasing the sensitivity of the asthma diagnosis (21). The diagnostic specificity was ensured by the research doctors making all diagnoses at the clinical research center minimizing the risk of misclassification by different doctors. Furthermore, the asthma diagnoses were strictly based on an algorithm including only children responding to inhaled corticosteroids and currently in need of this treatment after repeated attempts to withdraw the treatment (i.e., we have not included children with intermittent asthma in this end point definition). Other studies with less specific diagnostic criteria inevitably led to a higher prevalence of asthma. However, our approach with a strong emphasis on diagnostic specificity has proved powerful in both genetic (23, 26, 27) and clinical association studies (1, 2).

The high-risk nature of the cohort is a limitation to the generalizability of our findings and replication is needed in an unselected population-based cohort.

Meaning of the Study

It is intriguing that elevation of the biomarker u-EPX in healthy symptom-free newborns precedes the development of atopy later

in childhood. u-EPX is a biomarker known to correlate well with eosinophil count in blood and bronchoalveolar lavage fluid (28). Therefore, our findings suggest eosinophilic activation before atopy-related symptoms develop.

Elevated level of u-EPX in healthy asymptomatic neonates was associated with later development of allergic sensitization and eczema during the first 6 years of life. Previous studies have consistently reported increased u-EPX in preschoolers with established allergic sensitization (3, 5) and atopic dermatitis (4, 29), and in children more than 5 years of age with a diagnosis of atopic asthma (5, 6, 30, 31). We found no association between u-EPX at ages 1 or 6 months and the later development of troublesome lung symptoms or asthma, nor was u-EPX elevated in 6-month-old children with current troublesome lung symptoms, which is in agreement with a previous report of 1-year-old wheezy infants (32) and probably reflects the fact that airway

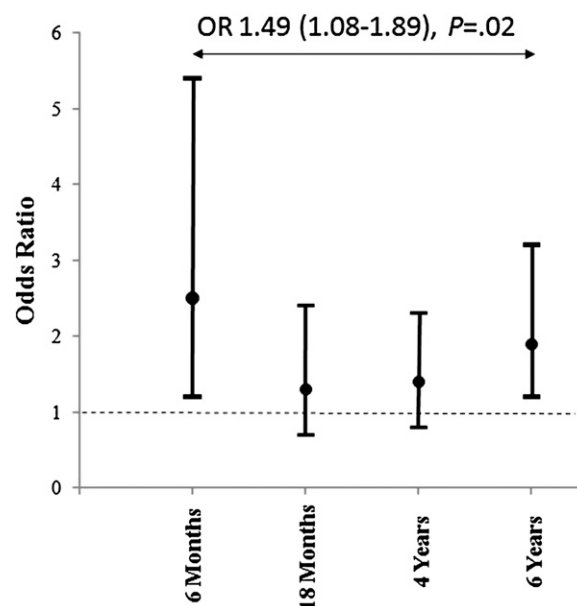


Figure 1. Association between urinary eosinophil protein X in healthy 1-month-old neonates and allergic sensitization during preschool age. The figure shows odds ratios of allergic sensitization at ages 6 and 18 months and 4 and 6 years (filled dot = odds ratio; vertical bar = 95% confidence interval) and the overall odds ratio calculated by a general estimating equation model. OR = odds ratio.

TABLE 3. ASSOCIATION AMONG URINE BIOMARKERS AT AGES 1 AND 6 MONTHS, BLOOD EOSINOPHIL COUNT AT AGE 6 MONTHS, AND THE DEVELOPMENT OF ECZEMA IN THE FIRST 6 YEARS OF LIFE

Inflammatory Markers	Eczema, 0–1 Yr		Eczema, 0–6 Yr	
	Hazard Ratio (95% CI)	P Value	Hazard Ratio (95% CI)	P Value
u-EPX, 1 mo	1.4 (1–2)	0.05	1.1 (0.8–1.5)	0.51
u-LT _{C4/D4/E4} , 1 mo	1 (0.8–1.2)	0.95	1 (0.8–1.1)	0.79
u-11 β -PGF _{2α} , 1 mo	1.1 (0.7–1.5)	0.74	1.1 (0.8–1.4)	0.59
u-EPX, 6 mo	1.5 (1.1–2)	0.02	1.3 (1–1.7)	0.04
Blood eosinophil count, 6 mo	1.2 (0.8–1.7)	0.31	1.2 (0.9–1.6)	0.19

Definition of abbreviations: CI = confidence interval; PG = prostaglandin; u-EPX = urinary eosinophil protein X; u-LT = urinary leukotriene.

eosinophils are scarce in this age group (33). In contrast, elevated EPX in nasal lavage samples from a population of healthy 4-week-old infants at high risk of atopy was significantly associated with the development of wheezing at age 6 months (34). However, this was a questionnaire-based survey with “a doctor’s diagnosis of wheezy bronchitis” as the main outcome measure and the authors found no association between EPX and parental observations of wheeze (34).

We also found a significant association between u-EPX at age 1 month and presence of nasal eosinophilia at 6 years of age, which has not been reported previously. Elevated u-EPX at ages 1 and 6 months showed increased odds of allergic rhinitis at age 6 years and the association was significant for the 6-month sample. A previous study found no association between u-EPX and allergic rhinitis (3), but this was based on questionnaire diagnosis in children at age 3 years (35 cases among 903 children) where a diagnosis of rhinitis is infrequent and difficult to establish (35).

The mechanism behind this presymptom eosinophilic activation is unknown. The origin of an early life eosinophilic activation might be dermal or nondermal (29). A dermal contribution to elevated u-EPX is suggested by increasing levels of u-EPX in children with increasing severity of atopic dermatitis (4). However, the gut mucosa has been proposed as a nondermal focus of eosinophilic inflammation in infants with food allergy (36), which is plausible because food sensitization prevails during infancy. Neonates with elevated u-EPX may host a distinct dysfunctional eosinophil cell immune phenotype with altered surface antibodies resulting in an increased liability to degranulate EPX and other eosinophil basic proteins. In addition, genetic variants in the eosinophil pathway activation genes might modulate immunoregulation (e.g., via IL-5, IL-10, IL-13, and IFN- γ) of eosinophil granulocytes (37, 38) and contribute to increased degranulation of EPX.

Blood eosinophil count at age 6 months was strongly associated with persistent blood eosinophilia until age 6 years, but interestingly, it showed poorer association with all other atopic end points than u-EPX at 1 and 6 months of age. This suggests that u-EPX is a better assessment of eosinophil activity than the blood cell count.

We found no association between u-LT_{C4/D4/E4} and u-11 β -PGF_{2 α} at age 1 month and any of the studied atopic end points. LTs and PGs are released during the immediate early phase allergic reaction by degranulation of IgE-sensitized mast cells, but are also released within the first hours of the late-phase reaction from several inflammatory cells types, such as mononuclear cells and basophilic granulocytes (39). In contrast, EPX is solely secreted by activated eosinophils, which are the predominant inflammatory cell type in the chronic late-phase reaction (16). The different cellular origin and temporal release pattern of EPX, LT_{C4/D4/E4}, and 11 β -PGF_{2 α} in the inflammatory cascade might explain why only EPX seems to play a role in the

inception of atopy very early in life. Future cellular studies of functional and regulatory aspects of the eosinophil granulocyte may help understand initiation of atopic disease early in life.

The clinical implication of our findings is that elevated u-EPX early in life seems to herald the onset of atopy-related conditions, such as allergic sensitization to food and inhaled allergens, allergic rhinitis, nasal eosinophilia, and eczema, but not troublesome lung symptoms or asthma during preschool age. The lack of association with preschool troublesome lung symptoms may reflect the heterogeneity of symptom triggers in this age group where allergens serve a quantitatively minor role compared with triggers of troublesome lung symptoms and asthma later in life.

Conclusions

u-EPX measured in healthy asymptomatic 1-month-old neonates was associated with the development of allergic sensitization, nasal eosinophilia, and eczema during preschool age. This suggests ongoing disease pathology including activation of eosinophil granulocytes preceding the development of childhood atopy-related disorders.

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