Eosinophil protein X and childhood asthma: A systematic review and meta-analysis

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Abstract
Background: There are no reference guidelines for health care providers regarding appropriate use and interpretation of urine eosinophil protein X (u-EPX) in clinical practice. Currently, there are no clear-cut clinical or laboratory parameters to diagnose asthma in young children. Objective: In this study, we (1) systematically reviewed and qualitatively appraised the epidemiological evidence to determine diagnostic u-EPX cut points for pediatric asthma, and (2) performed a meta-analysis to provide u-EPX estimates for diagnosing pediatric asthma. Methods: Research articles in literature were identified from PubMed/Medline and Web of Science databases from 1966 to August 2015. Children <18 years of age were included. Both serum and urine EPX were included. Twenty-seven studies met the inclusion criteria for the systematic review and nine studies for the meta-analysis. Details regarding EPX analyses, treatment efficacy, and outcomes were assessed. For meta-analyses, effect estimates were abstracted using standardized means. Results: Over 70% of studies found a significant relationship between u-EPX and childhood asthma. There was 1.94 times higher standardized means of u-EPX among acute asthmatics compared to healthy controls (confidence interval [CI]: 1.67–2.22). Similarly, the difference in standardized means between asymptomatic asthmatics and healthy controls was 1.58 times higher (CI: 1.27–1.88). Conclusions and Clinical Relevance: Despite differences in sample sizes, EPX processing and measurement, and ages of children, a consistent trend of higher EPX levels with childhood asthma was revealed.

Background
Childhood asthma

Asthma is a clinical syndrome characterized by airway inflammation and hyper-responsiveness, often triggered by respiratory infections, inhaled allergens, cold, wind, or dust [1]. It is the most common chronic condition in children [2]. In the United States, more than 10 million children under age 18 have been diagnosed with asthma [3]. This disease is the third-ranking cause of hospitalizations in children [4], and accounts for approximately 175,000 annual hospitalizations [5]. In the United States, the national annual healthcare cost for pediatric asthma is approximately $3 billion [6]. Problems with managing childhood asthma occur because of non-specific symptoms, as well as the inability to obtain reliable pulmonary function test (PFT) results, particularly in children below the age of 6 years [7].

Inflammatory markers

At this time, no single outcome provides a reliable measure for diagnosing asthma status. This is an area in which markers of inflammation may have a great impact, but their value in routine care has yet to be established. Currently, inflammatory markers do not appear to have a clinical role outside of research.

An ideal marker for identifying childhood asthma should (1) reflect spontaneous changes in disease activity, (2) reflect
improvements due to therapy, (3) have high sensitivity and specificity, (4) have prognostic significance, (5) be reproducible in steady state, (6) be fast, easy to obtain, and non-invasive, and (7) be inexpensive [8]. Several inflammatory markers for asthma have been tested in the past decade, including serum ECP, exhaled nitric oxide, and C reactive protein.

An inflammatory marker that is easy to collect in young children is vitally important. Of the four basic eosinophil granule proteins (i.e., major basic protein, eosinophil cationic protein, eosinophil protein X [EPX], and eosinophil peroxidase), EPX, also known as eosinophil-derived neurotoxin (EDN), is the only marker that can be accurately measured in urine [9]. However, the precise role of urinary eosinophil protein X (u-EPX) in the diagnosis of asthmatic disease remains controversial [10].

**Eosinophil protein X**

EPX may be the most valuable biomarker of eosinophil activation in childhood asthma [11] because it is sensitive, non-invasive, and easily measured in urine. Urinary eosinophil protein X (u-EPX) correlates inversely with nocturnal peak expiratory flow rates (PEFRs) and 1-second forced expiratory volume, reflecting its role as a marker in asthma activity [11]. u-EPX does not undergo significant in vivo degradation and is not influenced by perennial allergy and polysensitization [12] and can differentiate symptomatic from asymptomatic patients.

There is a much higher release of EPX than serum eosinophil cationic protein (s-ECP) in asthmatics, perhaps, indicating that eosinophils selectively release their granule proteins depending upon the type of stimulus to which the cells are exposed [13]. Thus, the determination of EPX in urine gives the same, if not better, information on disease activity and impact of treatment on inflammation as s-ECP.

Currently, normal reference ranges for u-EPX in children with asthma and healthy controls are lacking. Hence, the objective of this meta-analysis is to determine whether u-EPX is a valuable marker in diagnosing childhood asthma. Additionally, preliminary cut points will be proposed for EPX levels in asthmatics and healthy children. Both of these aims will be useful in guiding clinical practice for pediatric asthma.

**Methods**

**Data sources, search strategy, and study selection**

A systematic literature search of PubMed/Medline and Web of Science core collection databases were performed for studies published until August 2015, using the search terms, *childhood asthma*, *pediatric asthma*, *serum EPX*, *urine EPX*, *Eosinophil protein X*, *Eosinophil-derived neurotoxin*, *EDN*, and *inflammatory markers*. The search strategies are reported in detail in the flowchart in Figure 1.

Using the title and abstract, the literature search was reviewed independently in quadruplicate (H. Klonoff-Cohen, J. Lee, O. Refugio, M. Polvarapu) and screened against the study selection criteria to identify potentially relevant studies for full review. After the removal of duplicates, a total of 1191 articles were found from electronic databases, and from reference mining. Searches of bibliographies were also conducted to ascertain additional studies. Relevant studies were selected, assessed and data were extracted independently by four people (H. Klonoff-Cohen, J. Lee, O. Refugio, and M. Polvarapu). Disagreement was resolved by discussion and mutual agreement among the reviewers. Of the 1191 articles identified, 1128 articles were excluded based on the article’s title and abstract (not relevant), and 63 articles were retrieved for full text review. After complete assessment, only 27 articles met the established inclusion criteria. Authors of these 27 articles were contacted at least three times to obtain missing information.

**Eligibility criteria**

The following inclusion criteria were used for the systematic review: (1) Peer reviewed articles written in English; (2) cohort studies, cross-sectional studies, and case–control studies; (3) asthma as the index disease; (4) study sample: Children up to 17 years of age; (5) urinary and/or serum EPX used as a biological marker; (6) assessment of EPX in asthmatics and healthy control children. Review studies were excluded. Additionally, one randomized clinical trial was excluded because of the design. In this study, asthma patients were randomly assigned deliberate doses of montelukast in order to understand the variation in eosinophilic protein markers with medication. In all other studies, patients received treatments based on physician prescribed medications rather than being randomly allocated to pre-defined treatment groups. Hence, a total of 27 studies that complied with the pre-stated inclusion criteria were identified.

Among the 27 selected studies, for quantitative analysis (meta-analysis), the criteria used for inclusion were (1) presence of control group in the study consisting of healthy non-asthmatic children; (2) study group consisting of acute asthmatics OR asymptomatic asthmatics during the collection of urine sample; (3) reported values of u-EPX among asthmatics and healthy controls with measures of center and spread (mean/median; standard deviation/interquartile range); and (4) for a valid comparison, all the values of u-EPX reported in units μg/mmol Creatinine.
Data extraction

The results from studies that met the inclusion criteria and contained the outcomes of interest were included in the meta-analyses. All data were double entered and triple checked.

Summary outcome measures consisting of urinary EPX levels for childhood asthmatics/non-asthmatics, symptomatic/asymptomatic asthmatics, those on medication/without medications, atopic asthmatics/non-atopic asthmatics, as well as children experiencing acute exacerbations were extracted from studies and tabulated. Following data were extracted from the studies identified for this manuscript: author, publication year, study hypothesis, study design, sample size and characteristics of study and comparison groups, EPX measures, conclusion, and disadvantages of the studies. We also assessed the methodology used to measure u-EPX levels with the data, including test kit, detection limit/sensitivity of the kit, time and rate of centrifuging, freezer temperature, duration before freezing the sample, and any additional unique information provided pertaining to the process.

Assessing the quality of studies

The quality of each of study included in the quantitative analysis (meta-analysis) was assessed using the dichotomous criteria (Table 1) which was designed based on the “Quality Assessment Tool for observational cohort and cross-sectional studies,” “QUADAS 2,” and “Newcastle-Ottawa scale.”
Statistical analyses

The meta-analysis was performed using Stata software. The effect size representing the difference in u-EPX levels between asthmatics and control group children were calculated using standardized mean differences (SMD), also referred to as Cohen’s d. For studies that did not report the measures as means and standard deviations, attempts to contact the authors for the actual data were unsuccessful. For uniform measures of u-EPX to compare, evidence based conversion formulas [14–16] were employed to calculate estimated means and standard deviations from the reported values (e.g., median values, quartiles, and ranges).

Results

Study characteristics

As of August 2015, there were 27 studies which investigated the relationship between EPX and childhood asthma. Six studies used serum measures of EPX [11, 17–21], 18 studies used urinary measures of EPX [22–39], and three studies reported both serum and urinary measures of EPX [10, 12, 40] in relation to asthmatic and non-asthmatic status. There were 13 longitudinal studies [10, 17, 18, 22–24, 26–28, 32–34, 39], nine cross-sectional studies [11, 12, 25, 30, 31, 35–38], and two case–control studies [19, 40] (Table 2). In three of the studies, among the participants recruited for cross-sectional data, some were followed longitudinally for a time period [20, 21, 29].

Sample size and age

The sample sizes and ages of children for all the studies are displayed in Table 3. Children’s ages varied greatly from birth to 18 years, and the sample sizes ranged from small (n = 14) to large sizes (n = 903). Sources of patients consisted of patients in the emergency room [33–35], children from outpatient departments [11, 12, 17, 20, 23, 25–27, 29, 30, 35, 38–40], children recruited from school [38], a mother–infant cohort [22], hospitalized inpatients [18], and birth cohorts [22, 23].

Diagnosis and treatment of asthma

More than one-half of the studies conducted at least one type of pulmonary test on young participants, including whole-body plethysmography [12, 24, 29], spirometry [10, 11, 17, 21, 28, 30–32, 35, 37, 39], PEFRs [21, 26, 36, 37, 39], bronchial hypersensitivity using methacholine challenge [12, 17, 28, 31, 32, 40], FE(NO) levels in a subsample [22, 30–32], FEV₁ [11, 17, 21, 27–31, 35, 37, 39, 40], and FEF 25–75% [11, 27].

Skin prick tests (SPT) were used in four studies [10, 12, 17, 18, 20, 24, 35] to test for atopy. Questionnaires on children’s wheezing were completed by parents [2, 36] or young patients [38]. In addition, seven studies used respiratory diary cards [11, 20, 28, 31, 32, 39, 40]. Only one study focused on quality of life, using the Pediatric Asthma Quality of Life questionnaire and International Study on Asthma and Allergy in Childhood questionnaire [36].

Measurement of EPX

Differences in urinary EPX levels could be influenced by the methodology employed for measuring urinary EPX. Table 3 summarizes the test kit, detection limits, centrifuge spinning information (for serum not urine), freezer temperature, and duration of time before freezing and other information.
Sampling method for urine samples were collected by parents using cellulose tissue sanitary towels put into diapers.

Circadian variation in the sample

The use of u-EPX as a marker of early inflammation was not supported with this sampling method.

No difference in u-EPX levels in infants with a history of wheezing or atopic heredity.

The use of u-EPX as a marker of early inflammation was not supported with this sampling method.

Table 2. Studies investigating eosinophil protein X (u-EPX, serum EPX) and childhood asthma.

| Author                          | Objective/hypothesis                                                                 | Study design    | Study sample     | Comparison group | Diagnosis of asthma                                                                 | Results (u-EPX μg/mmol Cr; serum EPX μg/L) | Conclusions                                                                                           | Disadvantages                                                                                     |
|---------------------------------|-------------------------------------------------------------------------------------|-----------------|------------------|------------------|-------------------------------------------------------------------------------------|--------------------------------------------|-------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|
| Carlstedt (2011), Sweden [22]   | To determine if u-EPX and exhaled nitric oxide (FeNO) are objective markers of early airway inflammation in infants. | Prospective cohort | 110 mother–infant pairs with infant age 2–6 months | Based on parents completed questionnaire and physicians' examination | No difference in u-EPX levels in infants with a history of wheezing or atopic heredity | u-EPX was not associated with an increased risk of developing asthma by age 7 years, odds ratio 1.99% CI 0.5–1.8, \( P = 0.99 \). | The use of u-EPX as a marker of early inflammation was not supported with this sampling method. | For generalization of results, high risk nature of cohort is a limitation. |
| Chawes (2011), Denmark [23]     | Elevated levels of u-EPX, uLT, and \( \Delta F_{\text{PGF}}_{2\alpha} \), early in life reflects pre-symptom disease activity preceding later development of atopic disease | Prospective     | 369 healthy at risk neonates (those born to mothers with asthma) | Based on physicians' objective assessment and analysis of symptom recordings completed by parents | u-EPX measured in healthy asymptomatic 1-month-old neonates was associated with development of allergic sensitization, nasal eosinophilia, and eczema during pre-school age but not asthma. | u-EPX level reflects the presence of atopy and associated symptoms. | Its role as an adjunct in the diagnosis and management of allergic airway disease in early childhood might be important. | | |
| Gore (2003), United Kingdom [24] | To investigate the relationship between u-EPX and clinical phenotypes suggestive of allergic diseases | Prospective     | 903 children at age 3 years, followed prospectively from birth | Based on physicians' objective assessment and analysis of symptom recordings completed by parents | Physician diagnosed asthma was strongly associated with u-EPX levels, with highest levels found in children receiving asthma medication \( P < 0.001 \). | Children receiving short-acting \( \beta \)-agonists had significantly higher u-EPX levels (median 93.1, 95% CI 79.5–109.0) compared with those who did not (70.5, 95% CI 67.0–74.3 \( P = 0.001 \) ). | u-EPX level reflects the presence of atopy and associated symptoms. | | |
| Gravesande (1999), Germany [25] | To evaluate the usefulness of EPX excretion in monitoring therapy in asthmatic children | Cross-sectional | 22 stable asthmatic children 21 chronic asthmatics; 7 acute asthmatics; age 3.6–16.1 years | 16 non-asthmatic, non-asthmatic controls of ages 4.4–18.8 years | Diagnosis of asthma (chronic vs. acute) was based upon a history of chest tightness, cough and dyspnea and a 12% improvement of FEV₁ and/or FVC after \( \beta \)-agonists were prescribed. | Significantly higher u-EPX in chronic asthmatics (mean 124.7 ± SD 84.6, \( P = 0.0099 \)) and in acute asthmatics (233.3 ± 174.5, \( P = 0.0006 \)) before treatment compared with non-asthmatic, non-asthmatic controls (53.4 ± 29.0). | u-EPX is a valid tool for monitoring the effects of anti-inflammatory therapy in asthmatic children. | | |
| Hovelastra (1996), Netherland [40] | To whether serum and urine concentrations of eosinophil protein X (EPX) are elevated in asthmatic children | Case-control | 22 children of age 4–14 years, having diagnosis of allergic | 17 healthy controls having no symptoms or signs | Based on American Thoracic Society criteria (higher serum EPX levels among asthmatics (median 9.45; IQR 6.2–12.7) when compared to eosinophil cell u-EPX could be a simple, \( \Delta F_{\text{PGF}}_{2\alpha} \), early in life reflects pre-symptom disease activity preceding later development of atopic disease. | Higher serum EPX levels among asthmatics (median 9.45; IQR 6.2–12.7) when compared to eosinophil cell u-EPX could be a simple, \( \Delta F_{\text{PGF}}_{2\alpha} \), early in life reflects pre-symptom disease activity preceding later development of atopic disease. | Effect of confounders like allergic diseases has not been accounted for. | | |

Continued
| Author | Objective/hypothesis | Study design | Study sample | Comparison group | Diagnosis of asthma | Results (u-EPX μg/mmol Cr; serum EPX μg/L) | Conclusions | Disadvantages |
|--------|---------------------|-------------|-------------|-----------------|--------------------|------------------------------------------|-------------|--------------|
| Kalaajieh (2002), Lebanon [26] | To determine the concentration u-EPX to predict the severity and activity of asthma in children | Longitudinal | 80 non-atopic asthmatic children aged 5–12 years | 25 healthy, age and sex matched controls | Based on medical history and complete physical examination at hospital Severity of asthma based on asthma score including clinical and physiological parameters | u-EPX levels were significantly higher in asthmatics, both during (139.6 ± 11.7) and after (66.5 ± 9.3) attacks. u-EPX concentrations were significantly higher in asthmatic children than controls (P < 0.001). Statistically significant concentrations of u-EPX in asthmatic children, especially during acute exacerbation Measurement of u-EPX concentration may be useful in quantifying bronchial inflammation, thus, may serve as a marker of severity of the disease exacerbation and also facilitate early diagnosis and staging of the disease | | |
| Kim (2007), Korea [17] | To evaluate the use of serum EPX as a marker of airway inflammation in asthma in the diagnosis and evaluation of the severity and bronchial hyper-responsiveness in childhood asthma | Longitudinal | 72 atopic asthma children aged 6–15 years36 children with non-atopic asthma | 43 age-matched healthy children | Current asthma: wheezing or cough (absence of cold) in the past 12 months and a physician’s diagnosis (any time in the patient’s history) The severity of asthma was defined based on episodes of wheezing per year, speech interruption, and nocturnal waking due to wheezing | Serum EPX levels were higher among atopic asthmatics (80.1 ± 3.4) compared to non-atopic asthmatics (60.4 ± 36.3), and controls (52.8 ± 34.4 (P < 0.001). Serum EPX did not differ among non-atopic asthmatics and controls. Serum EPX levels of children with severe asthma (96.8 ± 31.7) differed significantly from those of children with mild (61.6 ± 35.6, P < 0.001) or moderate (69.7 ± 32.6, P < 0.01) asthma There was no statistical difference between the EPX levels of the mild and moderate asthma groups | Serum EPX can be clinically useful in atopic asthma children: Serum EPX might be another supportive biomarker for the diagnosis of atopic asthma, evaluation of asthma severity, and assessment of bronchial hyper-responsiveness | | |

Table 2 (Continued)
| Author | Objective/hypothesis | Study design | Study sample | Comparison group | Diagnosis of asthma | Results (u-EPX μg/mmol Cr; serum EPX μg/L) | Conclusions | Disadvantages |
|--------|----------------------|--------------|--------------|------------------|---------------------|------------------------------------------|-------------|--------------|
| Kim (2010), Korea [18] | To evaluate the utility of the serum EPX as a marker of eosinophil degranulation and its possible correlation with disease severity in childhood asthma | Longitudinal | 43 children with asthma aged 1.4–5 years having acute asthmatic symptoms | 19 age matched normal controls without history of acute or chronic respiratory symptoms | Diagnosed based on the Global Initiative for Asthma Guidelines | Serum EPX levels were significantly higher among asthma group when compared to controls (median 20, IQR 13.8–38.3), during both the acute (80, 55.2–113.0) (P < 0.0001) and stable (42.9, 28.8–79.2) (P < 0.0001) phases, and were also significantly elevated when comparing the acute phase to the stable phase (P < .001). Within the asthma group, the serum EPX levels significantly correlated with clinical asthma scores (r = 0.850, P < 0.0001) | Serum EPX is a useful marker for identifying disease activity in asthmatic children | Serum EPX levels may better reflect disease severity than ECP levels or total eosinophil count | Selection of subjects is based on the need for hospitalization and thus the sample is not representative of all asthma patients |
| Koller (1999), Austria [12] | To investigate whether eosinophil granule proteins correlated and whether there is a relationship between disease activity, pulmonary function and bronchial hyper-reactivity | Cross-sectional | 28 children with mild to moderate, atopic bronchial asthma (mean age 11.1 ± 2.2 years) under current anti-asthmatic treatment | 11 healthy non-atopic and non-smoking smoking adults (mean age 23.5 ± 2.4 years) | Based on recurrent obstructive pulmonary symptoms that were reversible with β₂ agonists and after exclusion of other conditions | u-EPX levels significantly raised in asthmatic children (median 49.4; IQR 34.2–64.0) compared to healthy controls (16.5, 7.4–25.6) (P=0.0001) u-EPX were significantly higher in symptomatic patients than in asymptomatic patients (P < 0.03) | Eosinophils activation in mild to moderate asthma as reflected by serum and urine concentrations of EPX and ECP is related to disease activity and weakly, albeit significantly to pulmonary function. Supports the use of anti-inflammatory markers (serum EPX, serum ECP and u-EPX) in monitoring the asthmatic children than pulmonary function | Discontinuation of topical steroids 48 hours prior to the tests may not be sufficient to wear off the anti-inflammatory effect |
| Kristjansson (1996), Sweden [10] | To investigate increased amounts of eosinophil granulocyte proteins in urine and serum reflect ongoing asthmatic inflammation and whether decreasing values reflect successful treatment | Longitudinal | 12 children with atopic asthma aged 8.1–15.6 years | 9 children without any atopic or known other diseases aged 9.1 to 15.4 years | Grading of asthma severity based on frequency of symptoms and the treatment required Atopy was confirmed with a positive skin prick test and/or positive serum RAST and increased IgE | At baseline, u-EPX was significantly higher in children with atopic asthma (mean 116.6) than in the control subjects (mean, 43.0) (P = 0.004) In the asthma group, u-EPX decreased significantly after 3 months of treatment with budesonide (1.64–68.4) (P=0.005) | u-EPX and serum ECP levels are useful markers of eosinophil activation and ongoing inflammatory activity in children atopic asthma. These levels are significantly lowered with anti-inflammatory treatment with inhaled steroids | Discontinuation of topical steroids 48 hours prior to the tests may not be sufficient to wear off the anti-inflammatory effect |
| Author                      | Objective/hypothesis                                                                 | Study design                   | Study sample                                      | Comparison group                                      | Diagnosis of asthma                                      | Results (u-EPX µg/mmol Cr; serum EPX µg/L) | Conclusions                                                                 | Disadvantages                                                                 |
|-----------------------------|--------------------------------------------------------------------------------------|--------------------------------|---------------------------------------------------|-------------------------------------------------------|----------------------------------------------------------|--------------------------------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| Labbe (2001), France [27]   | To analyze the role of u-EPX as a biomarker of eosinophil activation in asthmatic children | Longitudinal                   | 88 asthmatic children of age 1.1–16.1 years       | 34 children without any respiratory or atopic disorders | Diagnosed by pediatric pulmonologist                     | At baseline, u-EPX is significantly higher in asthmatics children (171) than in control group (60) (P < 0.01) | Among asthmatics, there was no difference in u-EPX levels among atopic and non-atopic children | Measurement of u-EPX, a reliable, non-invasive, sensitive and reproducible method to assess bronchial inflammation |
| Lonnqvist (2001), Sweden [28]| To relate clinical symptoms and deterioration of childhood asthma to inflammatory markers, after withdrawal of inhaled corticosteroids | Longitudinal                   | 34 mild-to-moderate asthmatic children aged 9–16 years on budesonide treatment selected to continue or discontinue treatment | 16 age matched healthy controls | Baseline values of serum EPX and urine EPX were significantly lower in healthy controls compared to asthmatics (P < 0.001) | Lower baseline levels of u-EPX (126) were associated with lower risk of exacerbations after withdrawal of budesonide (RR = 0.44, P < 0.03) | Because of easy sampling, EPX can be preferred marker in distinguishing healthy children from those with airway inflammation when diagnosis is unclear due to vague and atypical symptoms |
| Lugosi (1997), Austria [29] | To determine clinical use of u-EPX in monitoring airway inflammation in childhood asthma | Cross-sectional followed by longitudinal follow-up for some of the participants | 80 children with bronchial asthma of age 10.1 ± 3.1 years | 24 healthy, age-matched controls, Age = 11 ± 3.9 years | Based on obstructive pulmonary symptoms which are reversible with β2 agonists | u-EPX levels were increased in asthmatic children (median 68.4) compared to healthy controls (median 35.3; Cr: P < 0.0001); u-EPX levels were higher in symptomatic (median 123.5) than in asymptomatic patients (median 48.9) (P < 0.0001) independent of treatment or atopy | Measurement of u-EPX can be an alternative to assess asthma activity in children aged less than 5 years |
| Mattes (1999), Germany [30] | To assess the relationship between u-EPX and other markers of                         | Cross-sectional                 | 25 children with stable asthma of ages 6–16 years on corticosteroids | 9 healthy controls without atopic disorders or sensitization to | Based on clinical symptoms (cough, wheeze, and/or dyspnea) and | u-EPX levels significantly higher in asthmatics (median 58.2, 90% CI 29.2–181.1) compared to healthy controls (median 13.5, range 68–435) | u-EPX levels are significantly correlated with exhaled NO levels in asthmatics |
Table 2. (Continued)

| Author | Objective/hypothesis | Study design | Study sample | Comparison group | Diagnosis of asthma | Results (u-EPX μg/mmol Cr; serum EPX μg/L) | Conclusions | Disadvantages |
|--------|----------------------|--------------|--------------|------------------|---------------------|---------------------------------------------|-------------|--------------|
| Nuijsink (2007), Netherlands [31] | To investigate the relationship between u-EPX and asthma symptoms | Cross-sectional | 180 atopic children of ages 6–16 years with moderately severe asthma | From medical records | u-EPX levels were measured as median 185 and range 2–3114. | u-EPX levels did not correlate with established markers of asthma severity and eosinophilic airway inflammation in atopic asthmatic children. u-EPX seems unlikely to be useful biomarker for monitoring asthma in an individual child. | Diurnal variability may have introduced scatter of u-EPX, thus weakening a possible correlation. |
| Nuijsink (2013), Netherlands [32] | Changes in u-EPX would be related to changes in eosinophilic airway inflammation | Longitudinal | 205 atopic asthmatic children of ages 6.4–16.8 years using inhaled fluticasone | From medical records | After 2 year treatment period, the geometric mean u-EPX significantly decreased from 159 to 104 (P < 0.001). No correlations were found between u-EPX and asthma symptoms. u-EPX levels were higher in patients with airway hyper-responsiveness (AHR) than in patients without AHR. | u-EPX seems unlikely to be useful biomarker for monitoring asthma in an individual child. | |
| Oymar (2001), Norway [35] | To determine the clinical value of measuring u-EPX in children with asthma and to evaluate the influence of atopy and airway infections | Cross-sectional | 170 children with asthma of ages 12–179 months | 64 healthy controls | Based on episodes of cough/wheeze (response to β2 agonists) persisting or recurring for at least 6 months Compared to healthy controls (median 54, IQR 40–89), u-EPX levels were elevated in children with acute asthma (median 132, IQR 77–195, P < 0.001) and chronic asthma (median 93, P < 0.01). Among acute and chronic asthmatics, atopic children had higher levels of u-EPX than non-atopics (P < 0.05). Among chronic asthmatics, u-EPX levels were similar in asymptomatic and symptomatic asthmatics. u-EPX may reflect differences in eosinophil involvement between children atopic and non-atopic asthma. However, the individual spread within groups and the influence of airway infection limits its clinical use in childhood asthma. u-EPX alone is not sufficient to describe the airway inflammation or symptom activity in the individual child. | Circadian variation in u-EPX has not been accounted for as the samples were distributed throughout 24-h period. |
| Oymar (2001), Norway [36] | To determine the role of u-EPX in the prediction of recurrent wheezing and allergic sensitization 20 months later | Longitudinal | 105 children of ages 1–12 months hospitalized for wheezing | | | Study demonstrated that u-EPX was not a predictive factor for recurrent wheezing (OR = 1, 95%CI = 0.99–1.01). | Both under- and over-reporting of symptoms because of recall bias and differences in threshold for symptom self-reporting |
| Oymar (2001), Norway [33] | To evaluate the ability of u-EPX and | Longitudinal | 32 children of ages 12–36 months | 20 healthy children of ages 10–51 months | | Results suggest a possible role for u-EPX in the prediction of asthma. | Study included only a small number of children, and larger |
Table 2. (Continued)

| Author | Objective/hypothesis | Study design | Study sample | Comparison group | Diagnosis of asthma | Results (u-EPX μg/mmol Cr; serum EPX μg/L) | Conclusions | Disadvantages |
|--------|----------------------|--------------|--------------|------------------|---------------------|------------------------------------------|-------------|--------------|
| Rao (1996), United Kingdom [11] | To assess the role of serum EPX and ECP levels as measures of airway inflammation in childhood asthma | Cross-sectional | 48 children of ages 5–10 years with moderately severe asthma | Diagnosed by physicians | serum EPX negatively correlated with FEV1 (r = -0.35, P = 0.01) and FEF25–75% (r = -0.36, P = 0.02) | No data pertaining to the levels of serum EPX are available in the study | Serum markers of eosinophils correlate with airway function in childhood asthma similar to adult asthma | Study and control groups are from different populations to be compared |
| Reichenberg (2000), Sweden [36] | To examine this relationship between asthma severity and u-EPX in children | Cross-sectional | 61 children of ages 7–9 years with asthma | Healthy children from Lugosi study | Median u-EPX among asthmatic children was 88.6 (95%CI 67.5–135.7) | Findings give no further support for applying u-EPX as a general measure of disease severity in childhood asthma | | |
| Remes (1998), Finland [19] | To determine the value of measuring serum EPX and ECP in diagnosing childhood asthma | Case-control | 36 asthmatic children of ages 7–12 years | 166 children without asthma | Diagnosed by pediatric allergists clinically and by objective tests | Serum EPX levels were higher in asthmatics not receiving anti-inflammatory therapy (median 59.9, IQR 36.9–99.2) compared to controls (median 26.2, IQR 19.2–40.1) (P < 0.001) | The presence of asthma raises serum levels of EPX and ECP in children | This study does not assess severity of asthma |
| Severien (2000), Germany [37] | To compare levels of urinary EPX and leukotriene E4 between children with stable atopic | Cross-sectional | 80 children of ages 10.5 yrs ± 2.5 years with asthma | 28 healthy controls matched for age and sex | Diagnosed by physician | u-EPX was significantly increased in asthmatic children (median 85.5, IQR 64–131.5, SD 76.2) compared to controls (median 48.5, IQR 43.2–90, SD 56.7) | Urinary EPX is a useful noninvasive marker of airway inflammation and can be helpful as a complementary test in guiding asthma | |
| Author                  | Objective/hypothesis                                                                 | Study design                      | Study sample                          | Comparison group                                      | Diagnosis of asthma                                                                 | Results (u-EPX mg/mmol Cr; serum EPX μg/L)                                                                 | Conclusions                                                                 | Disadvantages                                                                 |
|------------------------|--------------------------------------------------------------------------------------|-----------------------------------|---------------------------------------|-------------------------------------------------------|--------------------------------------------------------------------------------------|-------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| Tauber (2000),         | To evaluate the use of u-EPX in epidemiologic studies in identifying atopic and asthmatic children | Cross-sectional                   | 877 Austrian school children if age 10–12 years | Based on modified International Study on Asthma and Allergy in Childhood Questionnaire completed by parents | u-EPX levels were higher in children with physician-diagnosed asthma (median 142.8 μg/mmol Cr) compared to healthy controls (median 63.9) (p < 0.0001). The odds ratio for u-EPX levels over the 90th percentile was significantly elevated for asthma (6.10, 95%CI 2.44–15.24) | No differences in u-EPX between the group of mild and the group of moderate to severe asthmatic children | Great overlap between controls and symptomatic asthmatics reduces the sensitivity of u-EPX in determination of the prevalence of asthma in epidemiologic studies |                                                                              |
| Wojnarowski (1999),    | To study the relationship between levels inflammatory markers (EPX and ECP in urine and nasal fluid) and clinical severity of childhood asthma | Longitudinal                       | 14 children of age 7.01–15.08 years with mild persisting asthma | Based on National Heart, Lung, and Blood Institute Criteria | Mean u-EPX levels at the last visit before exacerbation was 46.4 (SD 28), and at first visit after exacerbation was 46.1 (SD 22.5). Mean EPX values before, at, and after exacerbation were not different from values of patients without exacerbation at any time point. For children treated with long acting β2 agonists there was no difference in u-EPX compared with children without this therapy. | Though an increase in inflammatory markers during an exacerbation is seen, there exists a great variability in ECP and EPX levels in each patient and no increase in u-EPX (any inflammatory markers) preceding an exacerbation |                                                                              |
| Zimmerman (1993),      | To examine serum EPX, ECP, and eosinophil counts to distinguish between symptomatic and asymptomatic asthma patients, independent of treatment | Cross-sectional followed by longitudinal follow-up for some of the participants | 34 asthmatic children of age 6–17 years | 13 age matched children with chronic urticaria but no asthma | Symptomatic asthma: cough, wheeze, or breathlessness, physical symptoms of rhonchi and decreased air entry on auscultation and at least 15% response to inhaled bronchodilator | Serum EPX levels were higher in symptomatic asthmatics (mean 54.4) compared to control group (mean 23.5). Serum EPX levels significantly decreased among symptomatic asthmatics following treatment (54.4 to 24.5, P < 0.001), while the change among asymptomatic children were not significant | Serum EPX levels were higher in symptomatic asthmatics compared to asymptomatic asthmatics |                                                                              |
| Zimmerman (1994),      | To determine relationship between serum EPX and ECP, and                              | Cross-sectional followed by longitudinal follow-up for some of the participants | 14 atopic asthmatic children of age <5 years | 13 non-atopic asthmatic children of age <5 years | At baseline, serum EPX were higher in atopic asthmatics (mean 69.0) than non-atopic asthmatics (mean 19.6) | Higher levels of serum EPX among atopic asthmatics compared to non-atopic asthmatics were observed only |                                                                              |                                                                              |
Twenty-two studies used Pharmacia’s radioimmunoassay [10–12, 19, 20, 21, 23–30, 33–40], and five studies used ELISA immunoassay (from Diagnostic Development, Uppsala, Sweden and Medical and Biological laboratories, Nagoya, Japan) [17, 18, 22, 31, 32]. Eleven studies froze their samples at −20°C [10, 24, 26, 27, 29, 31–35, 40], eight studies froze samples at −70°C [11, 12, 18, 19, 25, 30, 37, 39]. The duration before freezing was the only factor that differed considerably, and could account for some of the variation. Carlstedt et al. [22] kept their samples cold and froze them within 24 h. Gore et al., Kalaajieh et al., Oymar, and Oymar et al. froze their samples within 10 h [24, 26, 33, 35], while Tauber et al. froze samples within 3 h [38]. Chawes et al., Gravesande et al., Koller et al., Kristjansson et al., Lugosi et al., Mattes et al., Nuijsink et al., and Wojnarowski et al. froze their samples immediately [10, 12, 23, 25, 29, 30, 32, 39].

**Qualitative analysis: preliminary cut points of u-EPX**

Urine eosinophil protein X concentrations were correlated with a variety of outcomes (endpoints) (Table 4). For example, u-EPX concentrations were higher in all asthmatic children compared to controls [18, 21, 25–27, 29, 30, 33, 35–37, 40] and higher during attacks in atopic [10, 19, 20, 29, 32, 33, 35, 38] and non-atopic asthmatics [19, 20, 29, 33, 35, 38] compared to controls. Table 4 summarizes the reported values of EPX from all the included studies.

Studies reported levels of u-EPX in a variety of groups of asthmatic children (e.g., chronic asthmatics, chronic asymptomatic asthmatics, chronic symptomatic asthmatics, atopic asthma, atopic symptomatic asthma, atopic asymptomatic asthma etc.) which limited the comparison of u-EPX levels across the studies. Mean urinary EPX levels were comparable between the acute asthmatics groups of Kalaajieh (Mean 139.6 ± SD 11.7) [26], Lugosi (Estimated mean 134.27 ± SD 9.6) [29], and Oymar (Estimated mean 134.67 ± SD 8.7) [35]. Additionally, a group of three studies reported identical mean u-EPX levels among the healthy comparison groups including Kalaajieh (Mean 35.3 ± SD 6.2 μg/mmol Cr) [26], Lugosi (Estimated mean 37.13 ± SD 18.28 μg/mmol Cr) [29], and Kristjansson (Estimated mean 43 μg/mmol Cr) [10]. Mean u-EPX levels among the healthy controls reported by Lonnkvist (Estimated mean 75.75 ± 43.25 μg/mmol Cr) [28], and Koller (Estimated mean 16.5 ± SD 13.92 μg/mmol Cr) [12] were different from other studies.
| Author                      | Sample size | Age group | Test kit                                      | Detection limit | Spin down sample? | Freezer temperature (°C) | Duration before freezing Other info                                                                 |
|-----------------------------|-------------|-----------|-----------------------------------------------|-----------------|-------------------|--------------------------|-------------------------------------------------------------------------------------------------------|
| Carlstedt (2011) [22]       | 110         | 2-6 months| ELISA immunoassay (Diagnostics Development, Uppsala, Sweden) | <3 μg/L         |                    | 80                       | Tubes were kept cold and frozen within 24 h Urine collected by parents from sanitary towels made of cellulose tissue placed in diaper |
| Chawes (2011) [23]          | 369         | 0-7 years | Double-antibody immunoassay (RIA – Pharmacia, Uppsala, Sweden) | <3 μg/L         |                    | 80                       | Immediately Transferred to 3.6 mL Nunc tubes and aliquots stored without addition of any preservatives Diluted x11 in phosphate buffer |
| Gore (2003) [24]            | 903         | 3 years   | Specific RIA (Pharmacia Diagnostics AB)        | <3 μg/L         |                    | 20                       | Within 10 h Serum centrifuged twice at 1450g                                                        |
| Gravesande (1999) [25]      | 44          | 3-18 years| RIA (Pharmacia & Upjohn, Freiburg, Germany)   | <3 μg/L         |                    | 70                       | Immediately Serum centrifuged twice at 1450g                                                        |
| Hoekstra (1996) [40]         | 39          | 4-14 years| RIA (Pharmacia, Uppsala, Sweden)              | <3 μg/L         | Serum centrifuged  | 20                       | Serum centrifuged twice at 1450g                                                                  |
| Kalaajieh (2002) [26]       | 105         | 5-14 years| Specific RIA (Pharmacia, Uppsala, Sweden)     | <3 μg/L         |                    | 20                       | Within 10 h Serum centrifuged twice at 1450g                                                        |
| Kim (2007) [17]             | 151         | 6-15 years| EUSA kit (7630, MBL, Nagoya, Japan)           | <3 μg/L         | Serum centrifuged  | 70                       | Serum on micro well with anti-human EPX antibody                                                   |
| Kim (2010) [18]             | 62          | 1.4-5 years| EUSA (MBL, Woburn, MA)                       | <0.62 ng/ml     | Serum centrifuged  | 70                       | 1 h at 25°C Diluted 11x in phosphate buffer                                                        |
| Koller (1999) [12]           | 28          | 11.1 ± 2.2 years| Sensitivity RIA (Pharmacia Upjohn AB, Uppsala, Sweden) | <3 μg/L         |                    | 70                       | Immediately Serum centrifuged twice at 1350g                                                        |
| Kristjansson (1996) [10]     | 21          | 8.1-15.6 years| Specific competitive RIA (Pharmacia, Uppsala, Sweden) | <3 μg/L         |                    | 20                       | Immediately Serum centrifuged twice at 1350g                                                        |
| Labbe (2001) [27]            | 100         | 1.1-16.5 years| RIA (Pharmacia Diagnostics, Uppsala, Sweden) | <3 μg/L         |                    | 20                       | Diluted in phosphate buffer Serum centrifuged twice at 1350g                                       |
| Lennqvist (2001) [28]        | 50          | 9-16 years  | Specific RIA (Pharmacia & Upjohn)             | <3 μg/L         | Serum centrifuged  | 20                       | Serum centrifuged twice at 1350g                                                                  |
| Lugosi (1997) [29]           | 104         | 10.1 ± 3.1 years| Specific RIA (Pharmacia, Uppsala, Sweden)     | <3 μg/L         |                    | 20                       | Serum centrifuged twice at 1350g                                                                  |
| Mattes (1999) [30]           | 34          | 6-16 years  | Double antibody RIA (Pharmacia & Upjohn)      | <3 μg/L         | Serum centrifuged  | 70                       | Serum centrifuged twice at 1350g                                                                  |
| Nujsink (2007) [31]          | 180         | 6-16 years  | EUSA (MBL, Nakaku Nagoya, Japan)              | <0.62 μg/g/l     | Serum centrifuged  | 20                       | Serum centrifuged twice at 1350g                                                                  |
| Nujsink (2013) [32]          | 288         | 6-14-16.8 years| EUSA (MBL, Nakaku Nagoya, Japan)              | <0.62 μg/g/l     | Serum centrifuged  | 20                       | Serum centrifuged twice at 1350g                                                                  |
| Oymar (2001) [33]            | 52          | 10-51 months| Pharmacia Diagnostics Uppsala, Sweden         | <3 μg/L         |                    | 20                       | Serum centrifuged twice at 1350g                                                                  |
| Oymar (2001) [34]            | 105         | 1-12 months | Specific competitive RIA (Pharmacia, Uppsala, Sweden) | <3 μg/L         |                    | 20                       | Serum centrifuged twice at 1350g                                                                  |
| Oymar (2001) [35]            | 314         | 0-15 years  | RIA (Pharmacia & Upjohn AB, Uppsala, Sweden) | <3 μg/L         |                    | 20                       | Serum centrifuged twice at 1350g                                                                  |
| Rao (1996) [11]              | 48          | 5-10 years  | RIA (Pharmacia & Upjohn AB, Uppsala, Sweden) | <3 μg/L         | Centrifuged at     | 70                       | Serum centrifuged twice at 1350g                                                                  |
| Reichenberg (2000) [36]      | 85          | 7-9 years   | RIA (Pharmacia)                               | 38.5 μg/g/l      | Serum centrifuged  | 70                       | Serum allowed to dot 60-120 min                                                                   |
| Remedios (1998) [19]         | 235         | 7-12 years  | Pharmacia Diagnostics Uppsala, Sweden         | 38.5 μg/g/l      | Serum centrifuged  | 70                       | Serum allowed to dot 60-120 min                                                                   |

Continued
u-EPX levels were higher in acute asthmatics when compared to chronic asthmatics reported by Gravesande et al. (estimated mean 233.3 ± SD 174.5 vs. estimated mean 124.7 ± SD 84.6 μg/mmol Cr) [25] and Oymar (estimated mean 134.67 ± SD 87.41 vs. estimated mean 96 ± SD 76.3 μg/mmol Cr [35]. u-EPX was higher in symptomatic asthmatic compared to asymptomatic asthmatic children [26, 28, 29], independent of treatment or atopy [28, 29]. Similarly, elevated levels were seen in persistent atopic asthmatics versus persistent non-atopic asthmatics [33]. Labbe et al. and Lugosi et al. [27, 29] demonstrated that the levels of u-EPX are independent of the atopic status and are higher among asthmatics than non-asthmatics.

Eight studies did not support u-EPX as a marker of inflammation in childhood asthma for estimating the course of disease [11, 22, 31, 32, 34, 38, 39].

Treatment protocols affected urinary EPX levels differently in studies. The change in level of u-EPX with medications (anti-inflammatory) for asthma was demonstrated in nine studies [10, 20, 25, 27–30, 32, 37], but a clear pattern could not be established. For instance, in a study by Nuijsink et al. [31], u-EPX levels among asthmatics receiving different doses of fluticasone (200 and 500 μg/day) demonstrated varying u-EPX levels of Median 189 (Range 2–2828) and median 180 (range 10–3114) mg/mmol. This wide range of U-EPX levels makes it difficult to interpret the effect of different fluticasone doses on the u-EPX levels. Nuijsink’s studies [31, 32] used wheezing during the first year of life as an endpoint and determined that u-EPX was not predictive for recurrent wheezing. Gravesande et al. [25] acute asthmatic group on 3 months of treatment with budesonide (400–600 μg) had similar u-EPX levels (mean 75.8 ± SD 59.5 mg/mmol) as two other studies: (1) Kristjansson’s acute asthmatic group on 3 months of budesonide and β2 agonists treatment (mean 68.4, 95%CI 41.4–95.4) [10], and (2) Kalaajieh’s acute asthmatic group on 2 weeks of salbutamol and salmedarol (mean 66.5 ± SD 9.3) [26].

A total of nine studies used serum EPX levels as a measure of airway inflammation in asthma [10–12, 17–21, 4]. Rao et al. [11] did not report any numerical results and Kim [17] compared mild, moderate, and severe asthmatics but with no comparison group. Remes’s asthmatic group (estimated mean 64.23 μg/mmol) [19] was fairly similar to Zimmerman’s atopic asthmatic group (Mean 69 μg/mmol) [20]. Among the healthy control group, the central measures of serum EPX are comparable in the studies by Remes (estimated mean = 28.5 μg/mmol) [19], Kim (estimated mean = 24.03 μg/mmol) [18], Koller (estimated mean = 25.26 μg/mmol) [12], Kristjansson (estimated mean = 30.8 μg/mmol) [10], and Zimmerman (mean = 31.2 μg/mmol) [20].
| Study (Year) | Condition | Median | Range | IQR | Healthy controls |
|-------------|-----------|--------|-------|-----|------------------|
| **Asthmatics** |           |        |       |     |                  |
| **Asymptomatic** |           |        |       |     |                  |
| Carlstedt (2011) [22] | NA | NA | NA | NA | Median: 21.4 |
|                     |       |       |     |     | Range: 7.1–81.1 |
|                     |       |       |     |     | IQR: 15.5 |
| Chawes (2011) [23] | NA | NA | NA | NA |NA |
| Gore (1999) [24] | 87.78 (77.38–99.58) | NA | NA | NA | NA |
| Gravesande (1999) [25] | NA | NA | NA | NA | 2.33 ± 174.5 |
|                     |       |       |     |     | 12.7 ± 84.6 |
| Kalaajieh (2002) [26] | 66.5 ± 9.3 | NA | NA | NA | 139.6 ± 11.7 |
| Labbe (2001) [27] | Mild/moderate: 171 (146–196) | NA | NA | NA | NA |
| Lonnkvist (2001) [28] | NA | NA | NA | NA | 68 (31–204) |
| Lugosi (1997) [29] | 68.4 (41.5–115.0) | 48.9 (30.8–67.7) | 65.1 (37.7–118.0) | 86.0 (48.7–112.1) | 123.5 (86.5–192.8) | NA | 60 (44–76) |
| Mallet (1999) [30] | NA | 58.2 (29.2–181.1) | NA | NA | NA | NA |
| Nujsnik (2007) [31] | NA | 185.0 (2.0–314.0) | NA | NA | NA | NA |
| Nujsnik (2013) [32] | NA | 184 (2–314) | NA | NA | NA | NA |
| Oymar (2001) [33] | 120 (67–123) | NA | 173 (123–196) | 73 (46–105) | NA | NA |
| Oymar (2013) [34] | NA | NA | NA | NA | 132 (QR 77–195) |
| Oymar (2001) [35] | NA | Acute 155 (113–253) | Chronic 110 (65–162) | Acute 102 (56–168) | Chronic 60 (39–123) | 93 (QR 46–149) |
| Reichenberg (2000) [36] | 88.6 (67.5–135.7) | NA | NA | NA | NA |
| Severien (2000) [37] | 85.5 (64–131.5) | 76.2 | NA | NA | NA |
| Tauber (2000) [38] | NA | 89.6 (27.6–280.3) | 62.5 (22.2–174.8) | NA | NA |
| Wójnarowski (1999) [39] | NA | NA | NA | NA | NA |

**Studies reporting only serum EPX levels**

| Study (Year) | Condition | Median | Range | IQR |
|-------------|-----------|--------|-------|-----|
| Kim (2007) [17] | NA | NA | NA | NA |
| Kim (2010) [18] | NA | NA | NA | NA |
| Rao (1996) [11] | NA | NA | NA | NA |
| Remes (1996) [19] | NA | 47.4 (34.8–97.2) | Mean: 86.6 | NA | NA |

**Continued**
Table 4. (Continued)

| Studies reporting only urinary EPX levels | Asthmatics | Asymptomatic | Atopic | Non-atopic | Acute | Chronic | Healthy controls |
|------------------------------------------|------------|--------------|--------|------------|-------|---------|------------------|
| Zimmerman (1994) [20]                   | NA         | NA           | Mean 69.9 | NA         | NA    | NA      | NA               |
|                                         |            |              | Asymptomatic: 4.2 | NA         | NA    | NA      | NA               |
|                                         |            |              | Symptomatic: 89.3 | NA         | NA    | NA      | NA               |
| Zimmerman (1993) [21]                   | NA         | NA           | NA      | Mean 54.4 | Mean 35.3 | Mean 31.2 | NA               |
|                                         |            |              | NA      | NA         | NA    | NA      | NA               |

| Studies reporting both urinary and serum EPX levels | Asthmatics | Asymptomatic | Atopic | Non-atopic | Acute | Chronic | Healthy controls |
|-----------------------------------------------------|------------|--------------|--------|------------|-------|---------|------------------|
| Hoekstra et al. (1996) [40]                         | NA         | u-EPX 16.2 (91–200) serum EPX 31.8 (23.3–40.7) | NA      | NA         | NA    | NA      | u-EPX 5.5 (IQR 3.4–7.9) Serum EPX 15.4 (IQR 10.3–23) |
| Kolier et al. (1999) [12]                           | NA         | NA           | NA      | NA         | NA    | NA      | NA               |
| Kristjansson et al. (1996) [10]                     | NA         | NA           | NA      | NA         | NA    | NA      | Serum EPX 30.8 (22.1–39.5) u-EPX 43 (23.3–62.7) |

Note: *Represents values reported as “median (quartiles 1 and 3)”; †represents values as “mean ± standard deviation”; ‡represents values as “mean (95% confidence interval)”; §represents values as “median (90% confidence interval)”; ¶represents values as “median (range).”
Based on the qualitative results, we determined preliminary u-EPX cut-points for asthmatics and healthy controls as 134–140 μg/mmol for acute asthmatics; 65–75 μg/mmol Cr for acute asthmatics after treatment; and 56–61 μg/mmol Cr for healthy controls (based on five studies). However, these data were extremely limited and consisted of medians and quartiles, rather than means and standard deviations. Therefore, estimates of means (and SD) were calculated from reported medians (and quartiles).

**Quantitative analysis: diagnosing asthma with EPX**

Due to the difficulty of diagnosing pediatric asthma in young children, several studies used terms such as airway inflammation, wheezing, or upper respiratory infections to describe asthmatics, making it difficult to determine if there were consistent trends of u-EPX across these studies. Nine studies [25–30, 33, 35, 40] reporting the levels of u-EPX in uniformly defined asthmatic subgroups (i.e., acute asthmatics, asymptomatic asthmatics) and healthy controls were selected for meta-analysis. Seven [25–29, 33, 35] out of nine studies reported u-EPX levels for acute asthmatics and healthy controls, and five [26, 28–30, 40] out of nine studies reported u-EPX levels for asymptomatic asthmatics and healthy controls. One study, Severien et al. [37], which met the inclusion criteria for the quantitative analysis was not included in the meta-analysis because of a high mismatch between reported (in the article) and the estimated standard deviations.

The comparison of SMD between acute asthmatics and healthy controls, and asymptomatic (stable) asthma patients and healthy controls were performed and illustrated in Figures 2 and 3. Both forest plots indicate that net u-EPX levels among asthmatics (irrespective of symptoms) are higher than those of non-asthmatics. Comparing acute asthmatics and controls, the forest plot in Figure 2 suggests that, on average, the mean u-EPX level among asthmatics is 1.94 times higher than those in the healthy control group with a confidence interval of 1.67–2.22. Similarly, the difference in standardized means between asymptomatic asthmatics and controls (Fig. 3) showed 1.58 times higher levels among asymptomatics than in healthy controls (CI: 1.27–1.88).

![Figure 2. Forest plot showing standardized mean difference in urine eosinophil protein X (u-EPX) levels among acute asthmatics and healthy controls.](image-url)
Study quality assessment

The results of study quality assessment for the nine studies included in the quantitative analysis are reported in Table 5.

Conclusions

There are currently a total of 27 studies investigating the role of urinary and serum EPX in pediatric asthma. Despite differences in study design, study sample size and characteristics (e.g., age or sex-related differences), presence or absence of a comparison group, choice of accompanying laboratory (e.g., skin tests) and pulmonary tests, time of measurement (number of weeks prior to an exacerbation, pre- and/or post-measurement), diagnostic criteria for asthma (e.g., based on history of recurrent varying number of episodes of wheezing [mild 1–2, moderate 3–12, and severe >12] within the past 12 months, and a physical exam by a physician), and

Table 5. Evaluation of quality of studies included in the meta-analysis.

| Research question | Study population | Selection bias | Inclusion/exclusion criteria | Measurement of exposure | Index test | Outcome measures |
|-------------------|------------------|----------------|-----------------------------|------------------------|-----------|-----------------|
| Gravesande (1999) [25] | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Hoekstra (1996) [40] | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Kalaajieh (2002) [26] | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Labbe (2001) [27] | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Lonnkvist (2001) [28] | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Lugosi (1997) [29] | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Mattes (1999) [30] | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Oymar (2001) [35] | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| Oymar (2001) [33] | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
varying endpoints, 19 out of 27 studies [10, 12, 17–21, 24–30, 33, 35–37, 40] reported a statistically significant relationships between EPX and childhood asthma.

A meta-analysis was performed that reported u-EPX values for both asthmatic cases and healthy controls. From the meta-analysis of seven studies [25–29, 33, 35], it was determined that the overall SMD between acute asthmatics and non-asthmatic healthy controls was 1.94 (CI: 1.67–2.22). Additionally, the meta-analysis results of five studies [26, 28, 30, 40] revealed an overall SMD of 1.58 (CI: 1.27–1.88) between asymptomatic asthmatic cases and healthy controls. Hence, Urinary EPX is elevated in children with either symptomatic or asymptomatic asthma compared to controls.

We attempted to establish preliminary u-EPX cut-points. The estimated cut-points for acute asthmatics after treatment range from 65 to 75 µg/mmol Cr [10, 25, 26], with large standard deviations. For healthy controls, based on five studies, the u-EPX range was from 56 to 61 µg/mmol Cr [27, 33, 35, 37, 40]. However, there were three additional studies with ranges from 35 to 43 µg/mmol Cr [26, 27, 29]. There were three studies that had extremely close results for acute asthmatics—from 134 to 140 µg/mmol Cr [26, 27, 35]. However, there were four studies that did not reflect these values and had huge ranges and standard deviations: 103–233 µg/mmol Cr [25, 27, 28, 33]. Hence, these cut points are not definitive, but serve as a first step towards further research in the diagnosis of asthma over time. Translating the proposed EPX cut-points and values for diagnosing pediatric asthma into the clinical arena is not appropriate at this time. Accurate determination of cut points requires obtaining sensitivity and specificity measures for every study. At this time, there is no data in existence to assess sensitivity and specificity of u-EPX measurement for diagnosis of pediatric asthma.

Limitations

Although the present meta-analysis establishes an association between u-EPX and asthma and makes a case for clinical managing asthma, there remain a few limitations. Studies were conducted over 2 decades (from 1993 to 2015). This affected both the diagnostic criteria of childhood asthma as well as the methodologies used for estimating urine or serum EPX. Regardless, the diagnosis of asthma is exceedingly difficult to confirm in young children and infants, and usually requires years of follow-up. Different criteria were used among the different studies (e.g., American Thoracic Society). Furthermore, the classification of asthma into mild, moderate, or severe was determined through a variety of clinical tests including asthma scores, chest X-rays, measures of oxygen saturation, NO, and PEFR values.

Several studies were cross-sectional and did not assess asthma over time. There were differences in methodologies including equipment and testing for EPX (which can significantly alter the values of u-EPX and serum EPX) [41], and these measurements were not repeated post-treatment. Circadian rhythm (lowest levels at 7 PM and highest at 7 AM) may have introduced scatter of u-EPX [42], weakening possible correlations. There were also variations in performing PFTs (i.e., type, equipment) from one study to another.

Some studies included patients who were well-controlled with treatment, thereby underestimating EPX levels during exacerbations. A few studies used comparison groups such as population-based controls. There were also differences in sample selection, patient characteristics, and inclusion/exclusion criteria. Most studies were conducted in Europe on a small sample. All studies were based on whites, with no consideration of other racial/ethnic backgrounds.

Although u-EPX is affected by age, symptomatic versus asymptomatic asthma, atopy, inhaled steroids, and infections specific for bronchial asthma [18], none of the studies adjusted for these effects. Additional confounding factors, including emotional triggers and environmental tobacco smoke, were also never considered.

Future directions

To date, this is the first systematic review to implicate a useful role for urine EPX in childhood asthma, as well as suggest age-specific cut-points for urine EPX for asthmatics and healthy young children. Urinary EPX, a simple and non-invasive technique, could be invaluable for diagnosing future asthma in children. Despite the limitations of the studies, a consistent trend of higher EPX levels with asthma was revealed. These results are too premature to draw any firm or generalizable conclusions. The utility of u-EPX as a clinically relevant diagnostic metric in childhood asthma has yet to be established. Further research is required to replicate these findings and to validate this biomarker in a methodologically sound population-based prospective cohort study consisting of measuring u-EPX over several years in age-specific (e.g., 0–4, 5–11, >12 years [43]) children with newly diagnosed probable persistent asthma, stratified by severity (i.e., mild, moderate, and severe), prior to and after initiation of long-term medications as well as during exacerbations, until a definitive diagnosis of pediatric asthma is confirmed. This will delineate the relationship between u-EPX and the progression of asthma; thereby determining whether this biomarker is potentially useful in the clinical arena.

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