

Review Article

Eosinophil granule proteins in the assessment of airway inflammation in pediatric bronchial asthma

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Eosinophil granule proteins such as eosinophil cationic protein (ECP), eosinophil peroxidase (EPO) and eosinophil protein X (EPX) in serum and urine are indirect measures of eosinophil activity. The measures have been evaluated for prediction, diagnosis and monitoring of anti-inflammatory treatment modalities in children with asthma. Assessments in serum and urine are highly dependent on sampling procedures and must be performed under strictly controlled conditions using standardized sampling and laboratory procedures. The measures are influenced by circadian and seasonal variations. Measurement of the eosinophil granule proteins does not improve the predictive value of a family history of atopy. Due to insufficient sensitivity and specificity, the measures are not useful in the diagnosis of asthma in children, and the clinical use of eosinophil proteins in the individual child for assessment of asthma severity has not been sufficiently validated. Serum and urine eosinophil granule proteins, however, may be useful in extending our knowledge of suppressive effects on eosinophil activity of various doses, devices and administration regimens of inhaled glucocorticoids in children. Such evaluations may be performed in randomized, double-blind trials of well-defined age groups and they should include measures of compliance. One important aspect to look at would be the distinction between suppressive effects on eosinophil activity and clinically important anti-inflammatory effects. Considering the complexity of airway inflammation and the heterogeneity of childhood asthma, however, it may be too simplistic to look for a single measure of the inflammatory processes. In the future, perhaps, a combination of products of inflammatory cells may give more clinically relevant information with respect to prediction, diagnosis, monitoring and outcome of childhood asthma.

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Children with allergic asthma have similar inflammatory changes in the airways to those seen in adults with the condition. Essential features may be narrowing of the airways, loss of epithelium, and cellular infiltration dominated by mast cells and eosinophils (1–5). The eosinophil and its mediators are involved in bronchoconstriction, epithelial damage and bronchial hyperresponsiveness (6–9). They may be increased in numbers in bronchoalveolar lavage fluid and in bronchial tissue from adults

(10) and children with asthma (9). Direct *in vivo* studies of such features, however, involve invasive procedures such as bronchoscopy and bronchial biopsy and little data have been provided in children due to the difficulty in performing the procedures (11, 12). Consequently, eosinophil granule proteins in nasal secretions, sputum, and in blood and urine have attracted attention as indirect measures of airway inflammation (13–17). Nasal lavage and induced sputum concentrations may be assumed to reflect airway

pathology more accurately than serum or urine eosinophil granule protein concentrations (16); however, the methods for obtaining nasal secretions and induced sputum may be difficult to apply in research protocols as well as in clinical practice, and relatively little data are available in children (17–26). Therefore, the present paper mainly concentrates on reviewing our current knowledge of blood and urine eosinophil granule proteins as indirect measures of airway inflammation in children with asthma.

The eosinophil granule proteins

Activated eosinophils release four cationic proteins: eosinophil cationic protein (ECP), eosinophil protein X (EPX), eosinophil peroxidase (EPO) and major basic protein (MBP). Animal studies have found MBP and ECP to have cytotoxic effects in airway epithelium, and EPX, also called eosinophil-derived neurotoxin, to have neurotoxic effects similar to ECP (27, 28). Though it may be present in neutrophils ECP is not produced by neutrophils (29), and ECP, EPX and EPO are considered to be specific for the eosinophil, whereas MBP in addition to eosinophils is produced in basophils (29). In accord with this, focus has been on ECP, EPX and EPO as indirect measures of eosinophil activity and airway inflammation. While some have suggested that EPO may be a more sensitive measure than ECP (30) others have not been able to confirm this (31, 32). Rigorous comparisons of the measures, however, have not been performed. The available clinical studies of EPO in children are relatively few (23, 30–34) and in the following most of the data referred to are derived from studies of ECP and EPX.

Methodological aspects

The granule proteins may be measured in blood and urine by specific radioimmunoassays (35, 36). In serum, the levels are higher than in ethylenediaminetetraacetic acid (EDTA) – plasma that is due to a combination of *ex vivo* release from serum eosinophils when activated during the clotting process and EDTA de-activation of plasma eosinophils (37). It has been suggested that ECP concentrations in EDTA plasma may reflect circulating levels, whereas concentrations assessed in serum may reflect the releasability of the granule protein from the eosinophil (38). Indeed, it has been indicated that ECP and EPX assessed in serum to a great extent reflect spontaneous release during

the processing of blood samples (39). The amount of ECP and EPX released also depends on factors such as incubation temperature, the time before centrifugation, recovery time and the type of containers used for collection of blood (38, 39).

Though ECP and EPX are closely related molecules, EPX is 70% homologous to ECP, and though they both undergo renal excretion only EPX can be accurately and reproducibly measured in urine (35, 40, 41). Urine EPX depends on the amount derived from eosinophil turnover and leakage from bronchial epithelium after eosinophil degranulation.

A urinary tract infection or the presence of leukocytes must be ruled out before assessment, as infection will increase EPX levels (38). To standardize for variations in renal function some have recommended correction of urine EPX for creatinine excretion (38); however, rigorous comparisons with absolute values have not been reported.

So far, one-time blood and spot urine collections have been mainly used in clinical studies of ECP and EPX in children. Few of the studies gave information on the time of day at which sampling was performed (42–45) and many of them may have been influenced by marked circadian variations in the measures. We have recently reported serum ECP and EPX concentrations to be considerably higher during the night than during daytime with peak levels of ECP and EPX at 06.00 hours (both markers) and trough levels at 08.00 and 12.00 hours, respectively (46). Similarly, peak levels of urine EPX/creatinine were found in urine collected during the night, trough levels in 4-h fractioned urine collected during the afternoon. These results support preliminary reports of serum ECP in adults (47) and of urine EPX in children (15). Our data also seemed to indicate that spontaneous day-to-day variations in ECP and EPX may occur (46); however, there are no available data to further elucidate the suggestion. Given that circadian variations in ECP and EPX reflect variations in eosinophil production probably depending on endogenous cortisol secretion rhythms such variations should indeed be expected (47, 48). The activity of endogenous hormonal secretory patterns, however, may also relate to season of the year (49). In accord with this it was recently reported that in Finland where marked variations in the number of daylight hours occur between Spring and Autumn, serum ECP and serum and urine EPX were significantly reduced from May to November in healthy children (50).

Prediction of asthma

From a preventative point of view the possibility of predicting asthma in children may be important in as much as immune response modulations to prevent progression to persistent disease in children at risk may be evaluated (51, 52). Several studies, however, have found no predictive power of eosinophil protein assessments in wheezing infants with respect to development of asthma (52–54). Wheezing infants in whom ECP in nasal secretions (52) and in serum (53), and EPX in urine (54) had been assessed were followed up for 6 months (52), 20 months (53) or 3 years (54), and the diagnosis of asthma was verified by a physician (52, 53) or based on a questionnaire (54). On the other hand, studies using similar designs have suggested that elevated ECP levels in nasopharyngeal aspirates (52) or serum (26, 56, 57) may be predictive markers of chronic wheeze after 4 (55, 56), 12 (57) and 24 months (26) follow up. The discrepancies may have been caused by differences in population characteristics, confounding influences from sampling procedures and variations in the measures as discussed above. In none of the studies did the inflammation markers do better than a positive diagnosis of respiratory syncytial virus infection in a wheezing infant, which has been found to be highly associated with the development of asthma and allergic sensitization at 7 years of age (58). In the individual child, eosinophil granule proteins or any other currently available measures of inflammation seem not to be an improvement on the predictive value of a family history of atopy (59).

Diagnosis of asthma

Considering the role of eosinophilic inflammation in asthma it has been suggested that eosinophils or its granule proteins may be applicable in the diagnosis of asthma (13, 35, 60). Eosinophils, however, are not specific for airway inflammation. Activated eosinophils may be found in subjects without asthma (61) while they may not be detected in others considered to have clinically significant asthma symptoms (62). Serum ECP may be elevated in several other inflammatory conditions of the lower airways such as mycoplasma pneumonia, viral infections, unexplained cough and host defense deficiencies (63–65). Urine EPX has been found to be elevated in children with laryngitis, pharyngitis and pneumonia (66). Serum ECP and EPX were found to be insensitive measures of eosinophil activation in bronchial allergen provocation (67).

Significant differences in serum ECP and EPX between atopic and healthy children have been detected; however, sensitivity and specificity of the measures are too low for diagnostic use in individual patients (13, 68, 69), and circulating eosinophil granule proteins have reproducibly been shown to reflect only the atopic state, not asthma (59, 70, 71). When the data are seen in the light of the current advocacy of the concept of inflammation being only one, though well documented, among several overlapping etiological factors with superimposed risk factors (72–74) it is, furthermore, clear that indirect measures of eosinophil granule proteins are not valid for the diagnosis of asthma in children.

Asthma severity

Serum and urine eosinophil granule proteins, however, could be of value in the clinical decision making on treatment strategies if they correlate with the severity of asthma (75). Studies looking at this have produced conflicting results. Some cross-sectional studies have found no correlations between serum ECP (42, 76), serum EPX (42) and lung symptoms, pulmonary function or bronchial responsiveness, others have found urine EPX to be weakly correlated with pulmonary function (14) and serum ECP to be correlated with symptom scores (77, 78) though not to pulmonary function (77). Longitudinal studies with observation periods of 1–6 (13), 6 (17) and 12 months (25) found only weak (13) or no correlation (17) between serum ECP (13) or urine EPX (17) and asthma symptom scores (13, 17) and pulmonary function (17). To some extent the conflicting results may have been caused by insensitive designs and heterogeneous and small study groups (14, 17, 42, 76, 78); however, it seems fair to say that the clinical use of eosinophil proteins in individual child for assessment of severity of asthma has not been validated.

Monitoring anti-inflammatory effects of inhaled glucocorticoids

Systemic glucocorticoids have been found to suppress serum ECP to normal levels and to cause concurrent improvement in pulmonary function (79, 80). The observations suggested that serum ECP and urine may be used for monitoring of the anti-inflammatory effect of topical glucocorticoids. Again, conflicting results have been presented in children (15, 25, 29, 35, 42–44, 79, 81, 82). Serum ECP was suppressed in children treated with inhaled budesonide

400–600 µg (81, 82), serum ECP, serum EPX and urine EPX in children treated with 400–800 µg daily (15, 35, 45). Others have reported that serum ECP (42, 43), and serum and urine EPX (42) levels were not affected in children during treatment with inhaled fluticasone propionate 200 µg (42) or budesonide 400–800 µg per day (43) in spite of increased pulmonary function and decreased bronchial hyperreactivity. Finally, serum ECP and urine EPX were not able to discriminate between the anti-inflammatory effect of 200 and 400 µg budesonide (44). No dose–response evaluations are available. Observation periods of 4 (44, 80) and 6 weeks (79), 3 (32, 42, 82) and 5 months (43) have been applied and heterogeneous groups with respect to asthma severity have been studied. In some of the studies the dose of the inhaled treatment varied during the observation period (79, 82). Few of the studies used randomized, double-blind designs (42, 44, 45). Most of the studies, furthermore, were flawed by the lack of measures of compliance (15, 29, 35, 42, 43, 79, 81, 82). Uncertainty about compliance is a major problem in clinical studies of the effect of inhaled glucocorticoids on inflammation markers (83). Equally important, perhaps, different age groups were studied. We recently found that serum ECP, serum EPX and urine EPX may be more sensitive measures of inflammatory effects of inhaled glucocorticoids in adolescents than in prepubertal children (45). This is probably due to an increase in systemic bioavailability of the inhaled therapy in combination with hormonal changes during puberty, which may increase clearance of the drugs. Recently, however, an open pilot study suggested that the finding of low serum levels of ECP may be a reliable marker for tapering off inhaled glucocorticoids (83). This has been supported in a double-blind, placebo-controlled longitudinal study that found that low baseline values of serum ECP and EPO, and urine EPX in children on inhaled budesonide were helpful in identifying the children in whom withdrawal of the inhaled therapy would be successful within a short-term perspective of 4 months (31). Evaluations of whether the measures in combination with other available clinical measures may be applicable for decisions about tapering off inhaled glucocorticoids in the individual child in the clinical setting, however, have not been provided.

Conclusions

Assessment of eosinophil granule proteins in serum and urine is highly dependent on sampling

procedures and must be performed under strictly controlled conditions using standardized sampling and laboratory procedures.

When planning clinical studies in children or when interpreting data from individual subjects steps should be taken to reduce confounding influences from circadian, seasonal and, possibly, day-to-day variations in the measures. To avoid confounding influences from circadian variations blood or urine should be sampled at consistent times. To reduce confounding from seasonal variations short-term clinical studies should preferably apply a cross-over design, and long-term studies (>3 months) should stratify for seasonal influences. The influence of spontaneous day-to-day variations may need further study.

In the individual child eosinophil granule proteins seem not to be an improvement on the predictive value of a family history of atopy. Due to insufficient sensitivity and specificity measures of serum and urine eosinophil granule proteins are not useful in the diagnosis of asthma in children, and the clinical use of eosinophil proteins in the individual child for assessment of severity of asthma has not been validated. Whether the serum or urine measures may be useful in the clinical guidance of tapering off anti-inflammatory treatment such as inhaled glucocorticoids remains to be finally settled. Facing the methodological problems in applying the measures in a clinical setting this may be considered to be difficult.

Serum and urine eosinophil granule proteins, however, may be useful in extending our knowledge of suppressive effects on eosinophil activity of various doses, devices and administration regimens of inhaled glucocorticoids in children. Such evaluations may be performed in randomized, double-blind trials of well-defined age groups and they should include measures of compliance. One important aspect to look at would be the distinction between suppressive effects on eosinophil activity and clinically important anti-inflammatory effects. Whether one of the available serum and urine measures of eosinophil granule proteins may be more sensitive for such assessments needs evaluation.

Finally, considering the complexity of airway inflammation and the heterogeneity of childhood asthma it may be too simplistic to look for a single measure of the inflammatory processes. Perhaps a combination of products of inflammatory cells may give more clinically relevant information with respect to prediction, diagnosis, monitoring and outcome of childhood asthma.

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