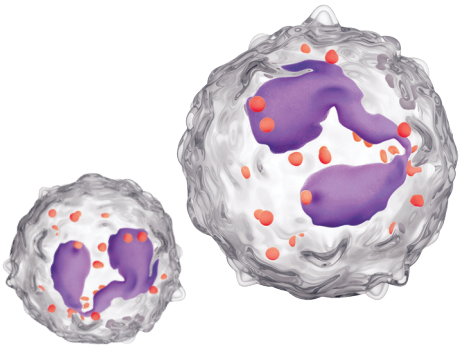
Eosinophil-Derived Neurotoxin



The role in asthma

Airway inflammation is established as the cornerstone of the pathophysiology of asthma. In this context, the eosinophil and its mediators have a multitude of proinflammatory functions, ranging from bronchoconstriction to epithelial damage and bronchial hyperresponsiveness.[1,2] Chronic inflammation in asthma involves both systemic and local eosinophilic inflammation. Eosinophils are increased in peripheral blood, sputum, bronchoalveolar lavage fluid, and in bronchial tissue from asthmatic subjects.[1,3-5] Eosinophils act as major effector cells through the release of granule proteins such as eosinophil-derived neurotoxin (EDN, also known as eosinophil protein X), which induces tissue damage and dysfunction.[6] Elevated levels of EDN reflect eosinophilic activity in the lungs and is associated with the severity and activity of asthma, as discussed below. In addition, following up the efficacy of anti-inflammatory therapies such as corticosteroids or biologics may be improved by measurement of EDN.

EDN

EDN has been identified as a cytotoxic secretory protein with anti-viral properties, which is released by degranulated eosinophils.[6] Compared to similar eosinophilic proteins (eosinophilic cationic protein [ECP], major basic protein [MBP], and eosinophil peroxidase [EPO]), EDN is significantly less charged and thus less sticky, making it more recoverable and easier to work with.[7,8] EDN can be measured in multiple specimen types, such as sputum, serum, urine, bronchoalveolar lavage fluid, and nasal lavage fluid.[9-13]

EDN is a stable analyte, which concentrations show low influence from circadian rhythm (contrary to blood eosinophils) or gender differences.[14] Smoking does not seem to affect EDN-concentrations in blood.[14]

Eosinophils are activated during blood clotting in serum tubes and thus release more EDN *ex vivo*, adding to the already existing EDN in the sample.[15] Measurement of ECP has shown both time and temperature dependency during serum sampling.[15,16] This effect seems less pronounced in the case of serum EDN.[15] Although most asthma studies of EDN have been performed in serum, EDTA plasma is an interesting alternative. The chelating agent EDTA binds Ca2+ needed for the EDN release from eosinophils.[15,16] Thus, an EDTA plasma sample may provide a better picture of the *in situ* concentration of circulating EDN in blood and the EDN concentration will be less dependent on time and temperature variation during sample preparation.

Serum or plasma EDN correlate to blood eosinophil counts but moderately to fractional exhaled nitric oxide (FeNO)[13] and likely reflect different aspects of asthma pathogenesis. EDN levels may therefore provide complementary information for diagnosis and to follow management of asthma when used together with FeNO. Measuring eosinophilic granule proteins may be more biologically relevant for asthma than counting blood eosinophils, since it is not necessarily the number of circulating eosinophils that matter, but rather their activation status.[10,17]

EDN in respiratory disease

EDN has been described as a promising marker for eosinophilic inflammation both in asthmatic patients and in wheezing children.[7,18] The presence of eosinophil degranulation products, including EDN, has been associated with allergic states of inflammation of the airway and with viral lower respiratory tract disease. EDN is associated with recurrent wheezing episodes after bronchiolitis, childhood and adult asthma, allergic rhinitis, aspirin-exacerbated respiratory disease, and eosinophilic chronic rhinosinusitis.[19-24] Blood levels of EDN seem to be correlated with atopy but also with asthma severity.[7,18]

EDN and asthma in children

Clinically, asthma in children below the age of 5 is underestimated because of a lack of diagnostic criteria.[25] In addition, lung function tests like the FeNO or spirometry tests are not adapted to small children for whom it is difficult to comply with a standardized single breath technique.[26]

In a study by Kim *et al.*, concentrations of serum EDN were measured in preschool asthmatic children during acute and asymptomatic periods of asthma and compared with EDN concentrations measured in healthy control preschool children.[20] Serum EDN levels were the highest in children with acute asthma, followed by those with stable asthma, and those in the control group, and the differences among the three groups were significant. Additionally, when asthmatic children were classified into mild, moderate, and severe subgroups based on asthma severity, the EDN levels were significantly different among the three groups, unlike the eosinophil counts.[20] Other studies indicate that a combination of eosinophil count and serum EDN measurement may better predict the risk of recurrent wheezing in early life.[27]

EDN has also shown promise in distinguishing wheezing children from children with pneumonia, common cold, or tonsillitis[28] and in aiding in the diagnosis of school age childhood asthma.[22]

Evaluation and monitoring of the inflammatory component of pediatric asthma may be essential to assess disease severity and need for treatment intervention. A randomized study used serum EDN as a biomarker for predicting responses to montelukast and budesonide treatment in symptomatic preschool children with asthma. The children were sensitized to house dust mites and had serum EDN levels ≥53 ng/mL.[29] During the study period, asthma control days increased in both groups. Serum EDN levels were significantly decreased in the montelukast group. The authors therefore recommended starting and stopping maintenance therapy with montelukast based on serum EDN concentration.[29]

EDN and asthma in adults

Eosinophilic inflammation is also a key component of adult asthma. EDN has been evaluated in several studies as an eosinophilic biomarker for assessment of the severity, asthma control status, and treatment response of adult asthma.

A study by Lee *et al.* investigated whether serum EDN was associated with asthma severity in adults (severe asthmatics, non-severe asthmatics, and healthy controls).[30] Serum EDN was significantly higher in severe asthmatics than in non-severe asthmatics and healthy controls. A significant correlation was observed between serum EDN levels and total eosinophil count. Thus, serum EDN level may be a useful biomarker for assessing asthma severity in adults.[30]

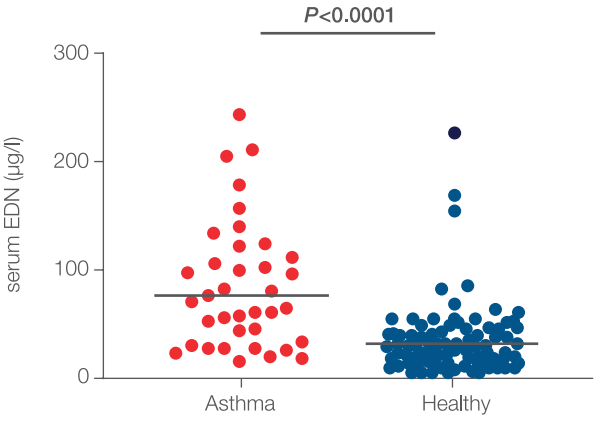
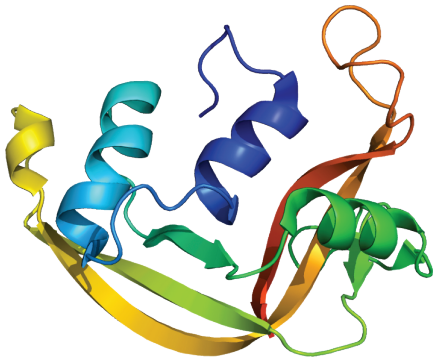


Figure. Serum EDN levels in asthmatic and healthy children (adapted from Rydell et al.[13])

Studies have also suggested that EDN may be a better indicator of asthma control status than blood eosinophil count.[17] The predictability of EDN for asthma control status was analyzed in uncontrolled asthmatics, controlled asthmatics, and healthy controls, and compared with blood eosinophil counts. Mean serum EDN levels in the uncontrolled asthma group were significantly higher than in the controlled asthma and healthy groups and correlated with blood eosinophil

counts. In the ROC analysis, serum EDN showed a significantly better performance than eosinophil count for predicting uncontrolled asthma status (AUC 0.726 vs 0.628, p=0.024).[17] Thus, serum EDN is a promising biomarker to differentiate between adult asthma patients with controlled and uncontrolled asthma. Furthermore, serum EDN may be a better biomarker for indicating asthma control status than total eosinophil count.[17]

In addition, with the development of new biologics, there is a need to follow the treatment response status in asthmatics who are being administered IgE- or eosinophil-targeting biologics. In a study by Gon *et al.*, a significant correlation was observed between a decrease in serum EDN level from baseline and lung function improvement after 8 weeks of omalizumab treatment (an anti-IgE monoclonal antibody [mAb]).[31] Likewise, benralizumab treatment (an anti-IL-5Rα mAb) reduced serum EDN levels compared to baseline[32] and EDN levels decreased significantly in patients with severe eosinophilic asthma after one month of reslizumab treatment (an anti-IL-5 mAb).[33] In the latter case, EDN levels correlated with eosinophil counts and the decrease in EDN levels were accompanied by an increase in FEV^1% after two months of treatment.[33] Thus, EDN appears to have potential as a biomarker for monitoring the treatment response to biologics targeting IgE or IL-5.



Conclusion

Studies clearly indicate a role for EDN in inflammatory diseases, including asthmatic inflammation. It has been shown that higher levels of EDN in asthmatics are concomitant with disease severity and related to treatment efficacy. Monitoring the efficacy of anti-inflammatory therapies such as corticosteroids and biologics may be improved by serial determinations of EDN levels in the blood.

As a treatment follow up tool, EDN has shown good results in children with asthma. In children too young to comply with lung function tests, EDN levels may be useful as an alternative measurement of eosinophilic inflammation. EDN can also be used in adult patients and in multiple specimen types (e.g. serum, urine, sputum, bronchoalveolar lavage fluid, and nasal lavage fluid). In conclusion, scientific literature suggests that EDN may become a novel biomarker for the diagnosis, treatment, and monitoring of asthma.

Product information

ImmunoCAP™ EDN Assay Kit, 10-9545-05

Note that the results generated from this ImmunoCAP test are intended for research and exploratory use only. Results cannot be used in diagnostics procedures.

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