

# Clinical Potential of Eosinophil-Derived Neurotoxin in Asthma Management



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The assessment and management of patients with asthma is challenging because of the complexity of the underlying inflammatory mechanisms and heterogeneity of their clinical presentation. Optimizing disease management requires therapy individualization that should rely on reliable biomarkers to unravel the phenotypes and endotypes of asthma. The secretory activity and turnover of eosinophils, as assessed by measuring eosinophil-derived proteins, may provide an accurate and complementary tool that mirrors the eosinophil activation status. Emerging evidence suggests that eosinophil-derived neurotoxin has considerable potential as a precision medicine biomarker. In this review, we explore the suitability of eosinophil-derived neurotoxin as a biomarker in asthma management, with particular emphasis on its clinical significance in the management of both pediatric and adult populations. © 2022 The Authors. Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>). (J Allergy Clin Immunol Pract 2023;11:750-61)

**Key words:** Asthma; Biomarker; Eosinophil-derived neurotoxin; Type 2 inflammation; Monitoring

## INTRODUCTION

Asthma affects all age groups and is a lifetime condition, thus posing an undisputable burden on the patients, their families, and the society.<sup>1-3</sup> Asthma is being increasingly deemed a significant global health issue,<sup>4</sup> with associated health care expenditures in developed countries being estimated at about 1% to 2% of the total health care costs,<sup>5</sup> with a mean cost per patient per year, including all patients with asthma (intermittent, mild, moderate, and severe asthma), ranging between US \$1900 in

Europe and US \$3100 in the United States.<sup>6</sup> Therefore, urgent diagnostic and therapeutic strategies as well as basic, clinical, and translational research-driven efforts are required to mitigate such burden, particularly those potentially addressing the heterogeneous and multifactorial nature of this disabling disease.

Asthma is a heterogeneous chronic airway inflammatory disease, with a wide range of clinical phenotypes<sup>7</sup> differing for exacerbation risk, response to treatment, and prognosis. Among asthma phenotypes, eosinophilic inflammation, triggered by allergen sensitization and T<sub>H</sub>2 lymphocyte-mediated immune response, is the hallmark of airway inflammation.<sup>8,9</sup>

Eosinophils contain approximately 200 granules/cell<sup>10</sup> and act as primary effector cells through the release of granule proteins such as the eosinophil cationic protein (ECP), major basic protein (MBP: MBP1, encoded by proteoglycan 2, and MBP2, a homolog of MBP1, which has been described as less cationic and for being strictly eosinophil-specific [because MBP1 is also present in basophils and mast cells]), eosinophil-derived neurotoxin (EDN), and eosinophil peroxidase. These proteins, which are all localized in the matrix of specific granules (except for MBP), exhibit cytotoxic and anti-infectious properties<sup>11</sup> through their ribonuclease (RNase) activity<sup>12</sup> and are involved in lung epithelium damage, mucus hypersecretion, airway remodeling, and inflammation.<sup>13-15</sup>

Phenotypic characteristics of patients with asthma may vary depending on the number of circulating eosinophils; for instance, subjects who display a high level of eosinophils are also characterized by higher IgE, lower FEV<sub>1</sub>, and an increase in risk of hospitalization.<sup>16</sup> However, it is acknowledged that there is a subgroup of adult patients with late-onset asthma that has high levels of eosinophils without being allergic.<sup>17</sup> Nevertheless, it has been increasingly recognized that eosinophil counts/percentages may provide only a limited understanding of the activity of these cells.<sup>15</sup> In addition, elevated eosinophil counts in peripheral blood in infants have been associated with subsequent

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ImmunoCAP assay for eosinophil cationic protein and has recently developed an ImmunoCAP assay for eosinophil-derived neurotoxin, which is currently available as a research use-only product. C.-K. Kim declares no conflicts of interest.

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*Abbreviations used*

*B-Eos*- blood eosinophil  
*COPD*- chronic obstructive pulmonary disease  
*DC*- dendritic cell  
*ECP*- eosinophil cationic protein  
*EDN*- eosinophil-derived neurotoxin  
*FENO*- fractional exhaled nitric oxide  
*ICS*- inhaled corticosteroid  
*MBP*- major basic protein  
*RNase*- ribonuclease  
*RSV*- respiratory syncytial virus  
*YKL-40*- chitinase-3-like protein 1

development of atopy in early childhood.<sup>18</sup> In contrast, the secretory activity and turnover of eosinophils, as assessed by measuring eosinophil-derived proteins, may provide a more accurate and complete picture as mirrors the eosinophil activation status.<sup>19-21</sup> Because EDN and ECP are released predominantly by eosinophils<sup>9</sup> (although EDN has also been localized to neutrophil granules<sup>22</sup>), any change in their levels would be a direct reflection of changes in eosinophilic inflammation. It is important to note that mostly ECP or EDN measured in serum is released *ex vivo* during the coagulation process after blood collection and reflects the activation level of eosinophils.<sup>23</sup> Accordingly, ECP and EDN were found to be more strongly associated with eosinophil airway inflammation, which contributes to bronchoconstriction and airway hyperresponsiveness, than was blood eosinophil (B-Eos).<sup>24,25</sup> In the future, studies aimed at examining the value of EDN and ECP as biomarkers to monitor asthma control would be desirable. B-Eos is an established marker of type 2 inflammation, reflecting IL-5–driven mechanisms, and is useful in selecting patients with severe asthma to be treated with anti-IL-5.<sup>26</sup> Another marker of type 2 inflammation, mainly reflecting activation of the IL-4/IL-13 pathway, is exhaled nitric oxide.<sup>27</sup> Information from both these markers can be combined for asthma diagnosis<sup>28</sup> and to obtain risk assessment in patients with asthma.<sup>29</sup> Some studies suggest that T2 inflammatory markers may work in synergy, because they have identified concomitant upregulation of T2 asthma biomarkers.<sup>30,31</sup> Accordingly, a recent pooled analysis of data stemming from several trials across the spectrum of asthma severity found that B-Eos and FENO provided additive prognostic information on asthma exacerbation risk.<sup>29</sup> However, some confounding factors, limited to suitability in underserved populations with asthma (eg, children aged <5 years), may lower their predictive value, thus suggesting the need of pursuing the search for additional reliable biomarkers with the aim to assist clinicians in asthma management.

Emerging evidence suggests that EDN has considerable potential as a precision medicine biomarker by acting as a surrogate marker of eosinophil activation. The clinical significance of EDN had been poorly explored until the development of ELISA, which has made its quantitative measurement more accessible to researchers.<sup>32</sup> Since then, mounting evidence supports EDN as a biologically and analytically attractive asthma biomarker in both children<sup>23,33,34</sup> and adults.<sup>20,35,36</sup>

In this narrative review, we provide a comprehensive overview of the scientific literature on EDN, with the purpose of assessing EDN in relation to the features that make it close to an ideal biomarker (eg, easy to obtain and store, readily quantifiable, and

stable for >1 year when frozen)<sup>36</sup> suitable for routine practice in the management of patients with asthma. We also discuss how the current knowledge may be harnessed from a diagnostic and therapeutic standpoint.

### Selection of evidence

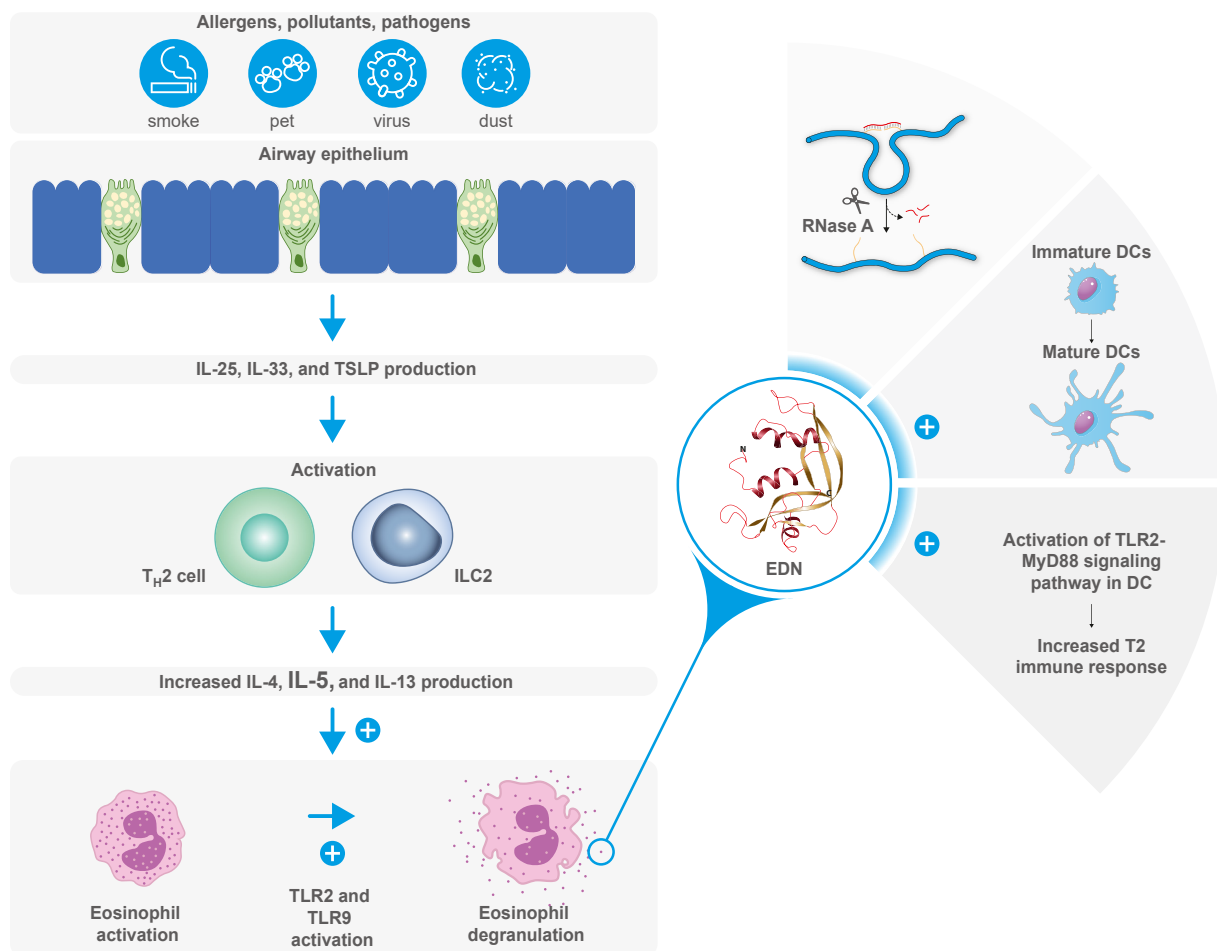
Articles considered for the present review were retrieved from a PubMed search completed in March 2022 and further updated in September 2022. The search terms were "eosinophil derived neurotoxin"[MeSH Terms] OR ("eosinophil derived"[All Fields] AND "neurotoxin"[All Fields]) OR "eosinophil derived neurotoxin"[All Fields] OR "eosinophil protein x"[All Fields] and "eosinophil derived neurotoxin"[MeSH Terms] OR ("eosinophil derived"[All Fields] AND "neurotoxin"[All Fields]) OR "eosinophil derived neurotoxin"[All Fields] OR "eosinophil protein x"[All Fields]. Articles were selected for inclusion according to their relevance for the topic, as judged by the authors, without limitations in terms of publication date and language.

## EDN BIOLOGICAL FUNCTION AND ITS SUITABILITY AS A BIOMARKER IN ASTHMA MANAGEMENT

EDN is a member of the RNase family, encoded in humans by the *RNASE2* gene, and represents the second most abundant protein in the human eosinophil proteome.<sup>37</sup> There is approximately 3.3 μg of EDN/10<sup>6</sup> eosinophils,<sup>38</sup> and EDN has also been isolated from various sources including placenta, liver, serum, plasma, urine, bronchoalveolar lavage fluid, and sputum.<sup>19,34,35,39-43</sup> EDN shares 67% amino acid sequence identity with ECP, but the ribonucleolytic activity of EDN is about 100-fold greater than that of ECP.<sup>44,45</sup> This enzymatic activity is a prerequisite for its cytotoxic, neurotoxic, and antiviral activities.<sup>44,46,47</sup> Quantifiable EDN levels are commonly measured in humans, thus suggesting that a constitutively low level may always be present, perhaps as a first line of defense against viral infection.<sup>48,49</sup>

Unlike the other eosinophil-derived granule proteins, EDN displays limited toxicity for helminth parasites and mammalian cells at high concentrations.<sup>11,50</sup> Importantly, EDN does not display a broad-spectrum antimicrobial activity and its release by eosinophils has been reported being bacteria-selective. To date, pathogenic bacteria such as *Clostridium difficile* and *Staphylococcus aureus* did induce EDN release, whereas the *Hemophilus* or *Prevotella* species did not.<sup>51,52</sup> As an RNase, EDN is considerably more effective against single-stranded RNA viruses such as the respiratory syncytial virus (RSV).<sup>46</sup> This activity may be targeted in asthma management because asthma exacerbations are commonly triggered by respiratory viruses.<sup>53</sup> In addition, recent evidence suggests that EDN has been uniquely upregulated in severe acute respiratory syndrome coronavirus 2 infection, and its release seems to occur in response to chemokines that have been detected in the airways of patients with this infection.<sup>54</sup> How eosinophils are regulated and when they are beneficial to the host with their antiviral activity and when they cause harmful inflammation are subjects for future research.

Dendritic cells (DCs) are the primary antigen-presenting cells and initiators of the immune response in allergic asthma,<sup>55</sup> and distinct DC subsets are involved in initiating and maintaining allergen-driven T2 immune responses in the airways. Therefore, DC maturation is key to attain an optimal T2 immunity.<sup>56,57</sup> EDN activates human DCs, leading to their expression of



**FIGURE 1.** EDN biological functions. *ILC2*, Type 2 innate lymphoid cell; *MyD88*, myeloid differentiation factor 88; *TSLP*, thymic stromal lymphopoeitin.

various inflammatory chemokines, cytokines, growth factors, and soluble receptors.<sup>48,58</sup> EDN also induces phenotypic and functional maturation of DCs, and it acts as an alarmin that activates the Toll-like receptor 2–myeloid differentiation factor 88 signaling pathway in DCs, enhancing T<sub>H</sub>2 immune responses.<sup>58</sup>

Collectively, EDN biological functions (Figure 1) and their relevance in the context of T<sub>H</sub>2 immune response and host response to viral infections commonly reported in asthma exacerbations highlight the clinical significance of EDN in asthma pathobiology.

An ideal biomarker should be able to identify the disease as well as the specific endotype/phenotype, useful in disease monitoring and prognosis evaluation, easy to obtain with minimum discomfort or risk to the patient, and easy to measure and store.<sup>59</sup> In addition, limited potential confounders are desirable because limitations of B-Eos are, for example, susceptible to smoking, circadian rhythm, and ethnicity.<sup>60,61</sup>

Mounting evidence supports the correlation of serum EDN with asthma disease severity<sup>35</sup> and with airway hyperreactivity.<sup>62</sup> It also supports the observation that patients with asthma display elevated levels of EDN, with higher levels being measured during asthma exacerbations when compared with levels in patients with stable asthma.<sup>15</sup> Furthermore, the predictive ability of serum

EDN levels to reflect the asthma control status has been recently explored.<sup>20</sup>

Asthma is a circadian disease, and so assessing circadian variability in biomarkers is paramount because the diurnal variability of any potential biomarker must be investigated in clinical practice. To date, it has been reported that the highest airway narrowing occurs overnight (with a peak at 4 AM) along with increased symptoms<sup>63</sup>; similarly, eosinophilic airway inflammation peaks in the early morning, with clinical implications for biomarker-guided steroid therapy.<sup>64</sup> Unlike commonly used asthma biomarkers, no circadian rhythm was identified for EDN,<sup>36</sup> thus simplifying the time of sampling and duration of storage for the specimens used to measure EDN.

Current cigarette smoking might impede the value of the biomarkers currently used for T<sub>H</sub>2-high inflammation.<sup>65</sup> Smoking influences eosinophil blood count and is associated with significantly lower levels of FENO.<sup>66</sup> Less is known regarding EDN, but no interference of smoking status with EDN levels has been reported in patients with asthma who have been on treatment for at least 12 months.<sup>36</sup>

A well-known phenomenon for protein and peptide biomarkers is the possibility of nonspecific binding to storage tubes; therefore, as a preanalytical step, it is paramount to minimize

events that may hamper a reliable analysis of biomarkers in human serum or plasma.<sup>67,68</sup> Unlike other eosinophil granule proteins, which are inherently sticky because of their strong polycationic properties, EDN is considerably less cationic than ECP (isoelectric point: 8.9 vs 10.8) and shows a negligible tube binding after incubation in different polypropylene storage tubes.<sup>36</sup> This feature favors sample preparation and minimizes the amount of protein that could be lost before analysis, thus ensuring an accurate blood concentration measurement. A recent study was carried out to assess the short- and long-term stability characteristics of EDN in whole blood and serum by resembling a real-world condition, including storing samples at room temperature, freezing them, and afterwards subjecting them to 3 repeated freeze/thaw cycles.<sup>36</sup> At room temperature, whole blood EDN levels were stable for up to 24 hours, which can be of high value from a practical standpoint when considering the time to transport and process venous blood into serum or plasma during clinical investigations. Refrigerated and frozen serums were also equally stable for up to 7 days and 1 year, respectively.<sup>36</sup> Not only blood storage conditions but also blood sampling procedures should be taken into consideration when assessing eosinophil granule protein concentrations. To date, temperature and duration of blood clotting, centrifugation, and hemolysis are the factors that may cause false-positive serum eosinophil granule protein levels. Sample blood clotting has been proposed to activate eosinophils. It thus makes them release more EDN into the serum, which would add to the free circulating EDN, similarly to what has been previously observed for ECP, although the effect seems less pronounced for EDN.<sup>34,69-71</sup> In a study by Rydell et al,<sup>34</sup> special attention was given to sampling, and the coagulation time was set to 60 to 90 minutes after blood sampling, on the basis of previous observations<sup>72</sup> for ECP, thus potentially minimizing artifactual increase in EDN levels. Importantly, although EDN can be measured reliably and robustly in both serum and plasma samples,<sup>34</sup> special attention should be given to the centrifugation procedure to minimize contamination of the plasma with white blood cell layer, which can be frequent in small-volume samples. Another desired characteristic of a biomarker is the easiness and noninvasiveness of assessment. To date, available EDN tests may require a smaller serum specimen volume from the patient, and this appears highly advantageous in young children because obtaining adequate specimen volume is sometimes prevented by a child's much lower total blood volume, difficulty in vein location, and their unwillingness to cooperate with the venipuncture procedure.<sup>21</sup> In addition, urine-based biomarkers are especially appealing in preschool children, because urine collection is noninvasive, easily accessible, abundant, stable, and comprehensive in metabolite composition.<sup>73,74</sup> Earlier studies suggested that measuring urinary EDN concentrations may serve as a sensitive, noninvasive technique in the evaluation of manifestations of airway inflammation.<sup>75</sup> A recent meta-analysis investigated the clinical significance of urine EDN as a diagnostic tool in childhood asthma and showed that urinary EDN concentrations are elevated in children with either symptomatic or asymptomatic asthma compared with those in controls.<sup>39</sup> Finally, mounting evidence suggests that nasosorption sampling may also be informative because this technique samples directly from the respiratory mucosa, thus not being contaminated by the saliva as occurs with breath or sputum sampling.<sup>76</sup> Importantly, nasosorption seems to be less invasive than the conventional swab and has been

found feasible in children with rhinitis, in neonates, and after nasal allergen challenge.

Overall, EDN may hold great promise as an easy, noninvasive biomarker, endowed with practical availability and reliability, whose clinical utility can be exploited in routine clinical practice. In the following paragraphs, we review the available evidence supporting the clinical significance of EDN in both pediatric and adult populations of patients with asthma and discuss how such knowledge can be harnessed from a diagnostic and therapeutic standpoint.

### EDN IN CHILDREN: A PROMISING BIOMARKER TO PREDICT WHEEZING, ASSESS DISEASE SEVERITY, AND MONITOR TREATMENT RESPONSE

The availability of noninvasive methods is of great value, particularly in children, for both diagnostic and monitoring purposes. We lack other clinically established, noninvasive or minimally invasive biomarkers of inflammation other than B-Eos in children younger than 5 years. Spirometry tests and FENO tests are not suitable for small children for whom it is difficult to comply with a standardized single-breath technique,<sup>77,78</sup> making these measures possible only from the age of 5 to 6 years. As a result, asthma in children younger than 5 years is associated with several clinical difficulties, including low accuracy in diagnosis, misevaluation of severity, and lack of objective data regarding therapeutic interventions.

Confirming asthma in preschool children is challenging because 1 in 2 children younger than 6 years experience wheezing, whose onset and persistence can be influenced by factors such as breast-feeding, atopy, indoor allergen exposure, environmental tobacco smoke, and viral infections. Respiratory tract virus infection (eg, RSV and rhinovirus) may cause symptomatic wheezing and asthma exacerbations.<sup>79</sup> Because EDN is implicated in antiviral activity against respiratory infections,<sup>46</sup> it has been investigated whether EDN might be one of the biomarkers for early identification of children at the highest risk of developing recurrent wheezing.<sup>33,80,81</sup> As shown in Table I, EDN was found to be significantly higher in infants with recurrent wheezing than in those without recurrent wheezing and correlated with the total number of wheezing episodes.<sup>81</sup> These results suggest that serum EDN levels are elevated in recurrent wheezing, asthma, and aggravation of disease, and that EDN may be an important biomarker for predicting recurrent wheezing.

Studies in children younger than 5 years suggested that EDN could be a useful biomarker for identifying disease activity in children with asthma and may reflect disease severity better than ECP levels or B-Eos would.<sup>21,83,84</sup> Because a clear differentiation between EDN levels in patients with asthma (in both acute and stable phases) when compared with those in controls could be detected, EDN may aid in the diagnosis of asthma. In children younger than 10 years, serum EDN levels were significantly higher in atopic patients with asthma than in nonatopic patients with asthma and in controls. In atopic and nonatopic asthma groups, serum EDN levels increased proportionally to disease severity. Of note, serum EDN levels were associated with bronchial hyperresponsiveness and proposed to aid in the diagnosis of asthma, especially atopic asthma, and in the evaluation of the severity and bronchial hyperresponsiveness in childhood asthma.<sup>85</sup> In children aged 10 years or older with bronchial

asthma, median serum EDN concentrations were significantly increased compared with those in healthy control subjects.<sup>86</sup> Furthermore, a study conducted in older children (age range: 6-17 years) undergoing a treatment showed that at baseline, EDN levels were significantly higher in patients with asthma than in control subjects (urticaria) while being reduced upon steroid therapy to a level like that seen in controls.<sup>87</sup> Identifying a correlation between biomarkers and parameters of disease severity may help to identify patients early and inform clinical decisions. As presented in [Table I](#), an earlier study in children younger than 5 years, who were divided into 3 subgroups on the basis of symptom scores, reported that only serum EDN levels were significantly different across subgroups.<sup>83</sup> Importantly, EDN levels differed significantly between disease phases (ie, acute vs stable) when compared with serum ECP levels and B-Eos.<sup>79</sup> The type of specimen may influence the correlation of EDN with markers of disease severity because at exacerbations a 4-fold increase in nasal EDN levels and a 1.6-fold increase in urinary EDN levels were observed.<sup>88</sup>

It has been suggested that B-Eos and FENO can be combined to achieve a risk assessment of children with asthma to identify those with the highest asthma morbidity.<sup>89</sup> Therefore, studies aimed at unveiling correlation between biomarkers may be of great value. A recent study using serum from children with asthma (aged 5-18 years) with physician-diagnosed asthma reported that EDN correlated well with blood eosinophil fraction but less with FENO; the latter possibly related to the fact that EDN and FENO reflect different aspects of asthma pathogenesis.<sup>34</sup>

The relevance of eosinophilic inflammation in asthma and its role across disease stages is perhaps best informed by response to therapies.<sup>8</sup> Therefore, the availability of a biomarker able to predict favorable patient response to therapies is of great value. In line with previous studies,<sup>83,90</sup> montelukast treatment significantly reduced serum EDN levels.<sup>91</sup> Importantly, treatment-related reduction in EDN levels may yield actual patient benefits because subjects receiving montelukast treatment over 12 weeks experienced a significant increase in the number of asthma control days (primary outcome). It is important to note that no significant change in serum EDN levels was reported among children receiving budesonide.<sup>91</sup> Overall, the clinical evidence currently available supports the notion that EDN can be endorsed as a valuable and useful test for childhood asthma to be evaluated in clinical practice for wheezing prediction, diagnosis, and treatment response in infants to adolescents ([Table I](#)).

### EDN IN ADULTS: A RELIABLE BIOMARKER OF DISEASE SEVERITY AND TO ASSESS DISEASE STATUS AND TREATMENT RESPONSE

As presented in [Table II](#), the clinical potential of EDN as a biomarker of disease and disease severity has also been explored in adult patients with asthma.<sup>34,92,93</sup> Earlier evidence was provided during the development of an ELISA method to measure EDN in multiple specimens, including serum, plasma, and urine.<sup>92</sup> Median EDN concentrations in serum, plasma, and urine were greater in asymptomatic patients with asthma compared with those in healthy control subjects,<sup>92</sup> as well as in symptomatic patients with asthma, namely, patients with mild to moderate asthma with a history of more than 300 eosinophils/ $\mu\text{L}$ .<sup>93</sup> The serum EDN level may be of help in assessing asthma severity in adult patients with asthma. Accordingly, the serum

EDN levels were found to be significantly higher in severe patients with asthma than in nonsevere patients with asthma and found to be better correlated with total eosinophil count than another biomarker of eosinophil inflammation such as periostin.<sup>35</sup> Higher levels of EDN related to poorer lung function (expressed as FEV<sub>1</sub>% predicted) were reported in adults with house dust mite allergic asthma, thus suggesting a relation between EDN and degree of airflow limitation.<sup>94</sup>

Some patients with asthma develop irreversible chronic airflow obstruction, so-called fixed airflow obstruction. Interestingly, a correlation between type 2 biomarkers and more asthma attacks has been reported, thus suggesting that identifying whether there is a relation between fixed airflow obstruction and type 2 inflammation markers could be important, because these markers of inflammation are regarded as treatable disease traits.<sup>95,96</sup> A cross-sectional study involving more than 400 patients with asthma showed that elevated urinary EDN levels were related to an increased likelihood of having fixed airflow obstruction, thus hypothesizing that this could possibly be targeted using eosinophil-directed therapies.<sup>95</sup>

Maintenance of well-controlled asthma results in better health outcomes<sup>97,98</sup>; therefore, monitoring control status using biomarkers of eosinophilic airway inflammation is relevant to inform therapeutic interventions. To this end, recent studies have tested the hypothesis that EDN levels may reflect a loss of asthma control associated with the progression of eosinophilic airway inflammation, thus underscoring the role as disease control marker for EDN ([Table II](#)).<sup>20,99</sup>

Monitoring asthma evolution over time is paramount to effectively follow guideline recommendations to review and adjust management of patients with asthma.<sup>100</sup> To this end, the availability of eosinophilic biomarkers positively correlated with symptom worsening could inform clinical decision and potentially identify subjects at higher risk of exacerbations. The association between EDN and various asthma characteristics in a large longitudinal asthma cohort has been recently investigated by taking advantage from the data collected from the Epidemiological Study on the Genetics and Environment of Asthma and reinforced the notion that EDN can serve as a potential biomarker to monitor asthma evolution in adults.<sup>92,99</sup> Importantly, among patients with asthma, high EDN levels were found to be associated with asthma attacks, wheezing and breathlessness, and use of asthma treatments.<sup>99</sup>

In the adult population, particularly smokers and older adults, distinguishing asthma from chronic obstructive pulmonary disease (COPD) can be challenging as well as diagnosing patients with both asthma and COPD.<sup>100</sup> A recent study enrolling patients with asthma, COPD, and exhibiting both conditions analyzed EDN levels and their potential correlation with previously documented markers of copresence of asthma and COPD, such as serum periostin and chitinase-3-like protein 1 (YKL-40).<sup>101</sup> As presented in [Table II](#), combined assessment of serum EDN and YKL-40 may have a potential clinical utility in identifying patients with asthma and patients with COPD as well as those presenting with both conditions with an eosinophilic phenotype, thus facilitating more targeted interventions for this challenging disorder. It is important to note that the diagnostic accuracy of using EDN was better than that of the combined assessment of serum periostin and YKL-40.

Inhaled corticosteroids (ICSs) remain the cornerstone of asthma management in adult populations. Because the clinical response to ICSs is often variable,<sup>102</sup> it is paramount for

**TABLE I.** Clinical potential of EDN as a biomarker in the management of children with asthma

Pediatric population	Biomarker definition according to BEST <sup>82</sup>	End points	Reference
Infants (mean age, 6-24 mo) hospitalized with their first episode of acute RSV bronchiolitis (n = 200) and treated for 3 mo with either montelukast or placebo	Predictive	EDN levels were higher compared with those in controls and correlated with the total number of wheezing episodes. The EDN cutoff value for predicting wheezing recurrence was 53 ng/mL. The positive predictive value of the 3-mo serum EDN level for the cumulative number of recurrent wheezing episodes at the 12-mo point was 57%; the negative predictive value was 76%; the sensitivity was 72%; and the specificity was 62%.	81
Children (age, ≥3 y) hospitalized with wheezing (n = 145)	Predictive	EDN serum levels were higher in infants with recurrent wheezing than in those without recurrent wheezing. In predicting recurrent wheezing, serum EDN showed the highest sensitivity (88.7%) and the lowest specificity (56.6%), with AUC of 0.795 ± 0.037.	80
Children (age, 0-7 y) with respiratory infections (n = 171)	Predictive	Serum EDN levels in the wheezing group were significantly higher than in the pneumonia, common cold, or tonsillitis subgroups (ie, nonwheezing group) ( $P < .001$ ).	33
Children (mean age, 2.9 y) hospitalized with acute asthma exacerbations (n = 43)	Diagnostic	Serum EDN levels were significantly higher in both acute (80 ng/mL) and stable (42.9 ng/mL) patients with asthma than in healthy controls (20 ng/mL) ( $P < .0001$ ). EDN levels correlated with symptom severity score at a greater extent than total eosinophil counts and ECP. The greatest correlation of serum EDN with symptom severity score ( $r = 0.850$ ; $P < .0001$ ) was observed during the acute phase. Using a cutoff value of 46 ng/mL for elevated EDN levels compared with those in controls, EDN levels could be predictive for asthma, with a positive predictive value of 93%, negative predictive value of 54%, sensitivity of 66%, and specificity of 89%.	83 84 21
Symptomatic children (mean age, 1.8 y) with atopy (n = 27)	Diagnostic	EDN levels were higher in atopic vs nonatopic children (69.0 vs 19.6 μg/L; $P < .01$ ).	
Children (mean age, 3.2 y) with physician-diagnosed asthma (n = 48)	Diagnostic	EDN levels were higher in children with asthma vs control children (77.33 vs 31.5 ng/mL; $P < .01$ ).	

(continued)

TABLE I. (Continued)

Pediatric population	Biomarker definition according to BEST <sup>82</sup>	End points	Reference
Children (mean age, 9.5 y) with atopic and nonatopic asthma and healthy controls (n = 151)	Diagnostic	Serum EDN levels were higher in atopic patients with asthma (80.1 ± 34.6 ng/L) vs nonatopic patients with asthma (60.4 ± 36.3 ng/L) and control children (52.8 ± 34.4 ng/L). Serum EDN levels were associated with bronchial hyperresponsiveness.	85
Children (mean age, 9.5 y) with bronchial asthma (n = 28)	Diagnostic	Serum EDN concentrations were significantly increased compared with those in healthy control subjects (74.8 vs 24.3 µg/L; <i>r</i> = 0.851; <i>P</i> < .0005). A significant relationship has been documented between serum EDN levels and B-Eos ( <i>r</i> = 0.851; <i>P</i> < .005).	86 88
Children (mean age, 11.7 y) with mild persistent asthma (n = 14)	Diagnostic	At exacerbations, EDN levels increased up to 4-fold in nasal lavage fluids (36.4 vs 141.7 µg/L) and urine samples (46.4 vs 74.1 µg/mmol creatinine).	
Children (age, 1-6 y) experiencing ≥3 recurrent wheezing episodes within 1 y and treated with either montelukast or budesonide for 12 wk (n = 43)	Response	Reduced serum EDN levels after montelukast treatment were associated with an increase in the number of asthma control days. Montelukast-related EDN reduction occurred only in those with baseline higher levels of EDN (>53 ng/mL).	91

AUC, Area under the curve; BEST, Biomarkers, EndpointS, and other Tools.

clinicians to rely on accurate biomarkers of ICS responsiveness. FENO is the biomarker reflecting IL-13–driven epithelial–inducible nitric oxide synthase production and can be used, to some extent, to assess response to ICSs and guide treatment.<sup>103</sup> Sputum eosinophils have been demonstrated to be able to guide ICS treatment, but their clinical use is hampered by methodological issues.<sup>97</sup> The ability of EDN to predict ICS responsiveness has not been assessed in current asthma management; however, there is evidence from several studies of reduced EDN levels after ICS treatment,<sup>104–106</sup> as presented in Table II. Overall, available evidence supports the notion that EDN can serve as a monitoring tool to assess ICS responsiveness in patients with asthma.

There is also a need for biomarkers to assess response to biological treatment in severe asthma. Of note, the targets of approved add-on biological treatments of severe asthma include, among others, IgE (eg, omalizumab) as well as IL-5 (eg, reslizumab) and its receptor (eg, benralizumab). A significant correlation between decrease in serum EDN levels from baseline and lung function improvement was reported after omalizumab, benralizumab, and reslizumab treatment,<sup>94,107,108</sup> which might be of relevance to assess treatment response to anti-IgE– and IL-5/IL-5R–targeted therapies.

A retrospective analysis of serum samples collected from adult patients with asthma provided further evidence that eosinophil depletion by biologicals could be associated with reduction in EDN levels.<sup>107</sup> In line with previous studies, patients with

asthma displayed higher serum EDN concentrations than did controls before receiving IL-5–targeted therapies, but experienced a significant reduction in serum EDN levels after benralizumab therapy. Of note, benralizumab did not modulate other inflammatory-related markers such as IFN- $\gamma$ , IL-17A, and IL-10, thus providing further insight into benralizumab's mechanism of action and its potential in mitigating eosinophil-mediated inflammation.<sup>107</sup> Finally, a recent prospectively designed real-world study investigated the changes in clinical parameters and serum biomarkers, including EDN, during 6 months of reslizumab treatment and suggested that changes in EDN levels may inform regarding therapy effectiveness before lung function improvement occurs.<sup>108</sup> Although serum EDN levels decreased with fluctuations during reslizumab treatment, they may still serve as a biomarker to monitor the degree of eosinophilic inflammation in patients with severe eosinophilic asthma.<sup>108</sup> Overall, available evidence suggests that monitoring EDN levels upon treatment, either ICS or IgE– and IL-5–targeted therapies, may assist clinicians in retrieving information regarding adult patients' response to therapy, although it remains to be established whether EDN measurements may guide treatment and improve long-term outcomes (Table II).

## CONCLUSIONS

The assessment and management of patients with asthma is challenging because of the complexity of the underlying

**TABLE II.** Clinical potential of EDN as a biomarker in the management of adults with asthma

Adult population	Biomarker definition according to BEST <sup>82</sup>	End points	Reference
Asymptomatic patients with asthma (median age, 38 y; n = 25)	Diagnostic	Median EDN concentrations in serum (36.9 vs 19.1 ng/mL), plasma (23.0 vs 14.5 ng/mL), and urine (118.2 vs 72.1 µg/mmol creatinine) were greater compared with those in healthy control subjects. EDN levels correlated well with B-Eos, but not serum IgE levels.	92 93 35
Mild to moderate patients with asthma with a history of >300 eosinophils/µL (n = 10)	Diagnostic	EDN concentrations in whole blood were also found to be significantly greater (31.7 ± 20.6 ng/mL) than in healthy volunteers (6.6 ± 3.0 ng/mL).	
Middle-aged patients with asthma (mean age, 46 y; n = 1133)	Diagnostic	Serum EDN levels were higher in severe patients with asthma than in nonsevere patients with asthma (69.08 ± 42.40 vs 58.46 ± 35.56 ng/mL; <i>P</i> < .05).	
Patients with asthma with controlled status (median age, 53 y; n = 56) and uncontrolled status (median age, 50.9 y; n = 75) and healthy controls (n = 43)	Monitoring	Serum EDN levels significantly differed between patients with controlled and uncontrolled status (60.8 ± 49.7 vs 103.2 ± 60.2 ng/mL; <i>P</i> < .01). EDN levels predicted asthma control better than total eosinophil counts (AUC, 0.726 vs 0.628; <i>P</i> = .024).	20 99
Never patients (mean age, 46 y; n = 567) and current patients with asthma (mean age, 39 y; n = 374)	Monitoring	Significant associations between both high ECP and EDN levels (as measured in plasma) and current asthma, asthma control, more frequent use of asthma treatment, and higher bronchial hyperresponsiveness in patients with asthma. High EDN level was associated with higher asthma attacks, worsening wheezing and breathlessness, and nocturnal chest tightness.	
Patients with asthma (mean age, 59 y; n = 177), COPD (mean age, 74 y; n = 61), and ACO (mean age, 70 y; n = 115)	Diagnostic	By using a cutoff level of 23.0 ng/mL, the proportion of patients with both high serum EDN and YKL-40 levels was greater in patients with asthma and COPD than in asthma or COPD subgroups (odds ratio, 3.85 [95% CI, 2.35-6.36]; <i>P</i> < .001; sensitivity, 45.2%; specificity, 82.4%).	101
Adults with asthma treated with prednisolone (n = 20)	Monitoring	Prednisolone treatment lowered EDN and ECP levels in both serum and bronchial wash.	104
Steroid-naive, nonsmoking adults with newly diagnosed asthma treated with fluticasone for 8 wk (mean age, 36 y; n = 16)	Monitoring	Both serum EDN and ECP levels were lowered by the 8-wk treatment with fluticasone while not correlating with reduction in bronchial nitric oxide flux.	105
Adults with allergic and nonallergic asthma treated with inhaled budesonide (high and low dose) or oral theophylline (n = 85)	Monitoring	Serum EDN levels were reduced by budesonide in a dose-dependent and temporally parallel fashion.	106
Adults with allergic asthma (n = 83) and positive for house dust mite-specific IgE (n = 40) and treated with omalizumab (n = 9)	Response	Lower serum EDN levels correlated significantly with the lung function improvement after omalizumab treatment as assessed by an increase in FEV <sub>1</sub> %. A significant correlation was observed between a decrease in serum EDN level from baseline and lung function improvement after 8 wk of omalizumab therapy ( <i>r</i> = -0.77; <i>P</i> = .015).	94

(continued)

TABLE II. (Continued)

Adult population	Biomarker definition according to BEST <sup>82</sup>	End points	Reference
Adult patients with asthma treated with benralizumab (age, 32–45 y; n = 9) or placebo (mean age, 37 y; n = 5)	Monitoring	Significant reduction in serum EDN levels (from 31.8 to 25.02 ng/mL) after benralizumab therapy compared with that in those receiving placebo (from 52.5 to 43 ng/mL).	<sup>107</sup>
Adult patients with asthma treated with reslizumab for 6 mo (mean age, 53 ± 13.9 y; n = 15)	Monitoring	After 1 mo of treatment, serum EDN and B-Eos levels were lowered, whereas FEV <sub>1</sub> improvement was documented after 2 mo of treatment. Patients with higher total eosinophil count/FENO level exhibited a greater reduction in serum EDN levels.	<sup>108</sup>

ACO, Asthma-COPD overlap; AUC, area under the curve; BEST, Biomarkers, EndpointS, and other Tools.

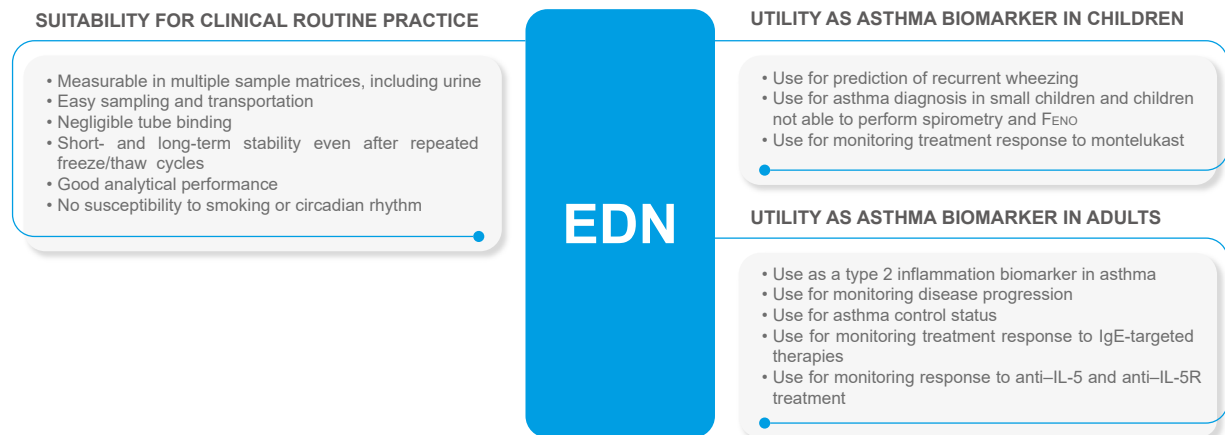


FIGURE 2. EDN is a clinically applicable biomarker in routine practice.

inflammatory mechanisms and the heterogeneity of their clinical presentation.<sup>109</sup> Optimizing disease management requires therapy individualization, which should rely on well-defined and reliable biomarkers to unravel the phenotypes and endotypes of asthma.

There is a quest for identifying more clinically useful biomarkers for asthma, and EDN may hold great promise as an easy, noninvasive biomarker, endowed with practical availability and reliability that may favor a feasible implementation into busy clinical practice. Overall, EDN may have a role in the stratification of patients according to the treatable trait of eosinophilic inflammation and as a possible guide for a more personalized therapeutic approach, thus aiding in disentangling this complex disease. It has the advantage that it can be assessed in different biological matrices, including urine,<sup>110</sup> and can also be used in small children. Nevertheless, clinicians should be aware that because of changes in sensitivity that have been developed over time, not all EDN immunoassays are equivalent. As a result, care should be taken when comparing published data sets. In addition, the advantage of monitoring EDN over other eosinophil-related biomarkers, including ECP, should be further assessed by taking advantage of the recently available automated assays that may provide more robust data than the previously used immunoassays. Further studies are necessary to address some unresolved issues regarding EDN, including the definition of reference values to allow for cutoff values for asthma diagnosis in

both children and adults, and additional prospective and longitudinal investigations are required to explore EDN potential predictive value for asthma exacerbations and assessment of adherence to treatment regarding asthma monitoring. Nevertheless, recent studies have highlighted the clinical value of EDN as a biomarker of eosinophilic inflammation and of screening, treatment, and monitoring<sup>111</sup> while serving as a useful predictor of asthma development in preschool wheezers.<sup>112</sup> Finally, exploring the value of EDN as a biomarker of airway inflammation may also hold promise; to this end, using nasal lavage or nasosorption sampling may be informative, particularly in the pediatric population from which nasosorption samples may be easier to obtain than blood samples.<sup>76</sup>

EDN is a bona fide biomarker, with potential for implementation in routine practice, and is reliable from a clinical standpoint by virtue of its technical features and monitoring tool capability along the journey of a patient with asthma (from diagnosis to follow-up treatment) and across age groups, thus serving as an additional option in the toolbox of clinicians dealing with patients with asthma (Figure 2).

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