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ORIGINAL ARTICLE

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Eosinophil-derived neurotoxin levels in early childhood and association with preschool asthma – A prospective observational study

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Abstract

Introduction: Eosinophil-derived neurotoxin (EDN) is related to childhood asthma, while normal values are lacking. We aimed to document serum EDN levels at 1 and 3 years in general and in non-atopic children, and explore if EDN levels differed by sex or were associated with preschool asthma at 3 years.

Methods: From the PreventADALL birth cohort, we included 1233 children with EDN analysed using ImmunoCAP at 1 and/or 3 years. Non-atopic children had no history of wheeze, asthma, allergic sensitization or atopic dermatitis. Preschool asthma was defined as having ≥3 episodes of bronchial obstruction between 2 and 3 years, plus doctor diagnosed asthma and/or asthma medication use by 3 years. The upper limit of normal (ULN) of EDN was defined as the 95th percentile. With Youden Index we calculated EDN cut-off levels for risk of preschool asthma.

Take home message 1: EDN levels were higher at 1 compared to at 3 years. **Take home message 2:** EDN levels at 1 and 3 years were overall higher in boys compared to girls, but remained similar between the sexes in non-atopic children at both ages. **Take home message 3:** Compared to non-atopic children, EDN levels at 1 and 3 years were higher in children with preschool asthma and other allergic diseases at 3 years. At 1 year, boys with allergic disease at 3 years more often had higher EDN levels compared to girls. **Take home message 4:** Higher EDN levels at 1 and 3 years were overall associated with preschool asthma and other allergic diseases at 3 years.

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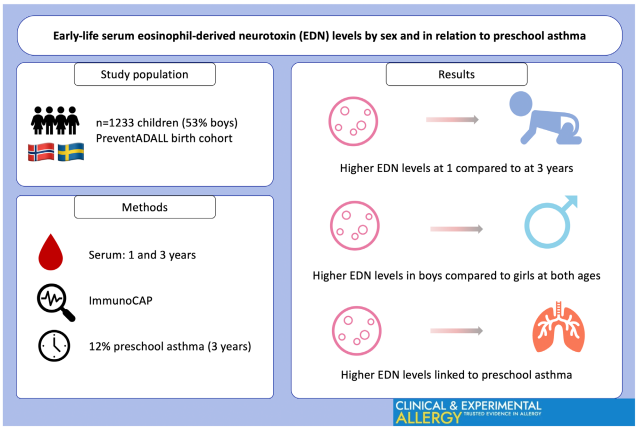
Results: The overall median (ULN) EDN levels were 27.4 (121) μg/L at 1 year ($n = 787$), and 20.1 (87.8) μg/L at 3 years ($n = 857$). Non-atopic children had EDN levels of 24.0 (107) μg/L at 1 year ($n = 147$), and 17.3 (84.6) μg/L at 3 years ($n = 173$). EDN levels were higher in boys compared to girls; 32.0 (133) versus 24.5 (97.0) μg/L at 1 year, and 20.9 (96.3) versus 19.0 (72.4) μg/L at 3 years. Preschool asthma was observed in 109/892 (12.2%) children. Higher EDN levels at 1 (>26.7 μg/L) and 3 (≥20.5 μg/L) years were associated with preschool asthma; adjusted OR (95% CI) 2.20 (1.09, 4.41) and 4.68 (2.29, 9.55), respectively.

Conclusion and Clinical Relevance: We report EDN values in early childhood, demonstrating higher levels at 1 compared to 3 years and in boys compared to girls at both ages. Higher EDN levels at both ages were associated with preschool asthma. However, EDN cut-off levels for preschool asthma were overall lower than the ULN of non-atopic children, limiting translation into clinical practice.

KEYWORDS
asthma, child, eosinophil-derived neurotoxin, PreventADALL, sex, type 2 inflammation, wheeze



GRAPHICAL ABSTRACT
In a paediatric population of 1233 children, serum eosinophil-derived neurotoxin (EDN) levels were higher at 1 compared to 3 years, and in boys compared to girls. Higher EDN levels at both ages were linked to preschool asthma.

1 | INTRODUCTION

Asthma is a chronic obstructive airway disease that often presents early in life,[1] affecting around 11% of preschool children.[2] Common features of asthma in early childhood include recurrent episodes of wheeze, coughing and chest tightness, often triggered by viral infections, physical activity, allergen exposure and other environmental factors.[3] Due to the heterogeneity of asthma and lack of objective tests to document the disease, the diagnostic criteria in young children are mainly based on patterns of symptoms, risk factors, response to treatment and exclusion of alternative diagnoses.[3]

Eosinophils play a key role in the development of asthma[4] and other allergic diseases.[5] Triggered by exposure to allergens or by infections, eosinophils in mucosal tissue are activated releasing pro-inflammatory degranulation products such as eosinophil-derived neurotoxin (EDN).[5] This eosinophilic-induced inflammation is also seen in asthmatic children,[4] causing damage and dysfunction of lung tissues leading to airway inflammation.[6] The

Key Messages

- Eosinophil-derived neurotoxin (EDN) levels were higher at 1 compared to at 3 years.
- Boys had higher EDN levels at both ages compared to girls.
- Elevated EDN levels in infancy was linked to preschool asthma.

biomarker EDN has no clear circadian rhythm and appears to display stable serum concentrations over time and at various storage temperatures.[7] EDN levels reflect eosinophil count[8–10] and likely also inflammation as elevated levels have been observed in tissues and body fluids weeks before asthmatic exacerbations manifest.[4,5] Compared to eosinophil count, EDN may be a better indicator of asthma control status.[11] Case–control studies have reported higher EDN levels in preschool- and school-aged children with asthma[9,12–17] and allergic asthma,[14,18] as well as in asthmatic children without continuous corticosteroid treatment.[16] In children with asthma, EDN was associated with respiratory symptoms,[14,15] allergic sensitization (AS),[19] atopic dermatitis (AD),[16] and correlated to symptom burden.[13,14,20] In children recovering from airway infections, EDN was higher among wheezing compared to non-wheezing children,[21] and correlated to future wheezing episodes.[22] Thus, EDN may be useful in diagnosis and management of childhood asthma,[4] but larger population-based studies are needed to confirm its potential role in clinical practice.

Although several case–control studies have established a link between EDN levels and various allergic diseases, normal values for children are lacking.[6] In adult populations, higher EDN levels have been observed in males compared to females,[8] but in children sex differences remains unexplored. Similarly, the relationship between early-life EDN levels in low-risk paediatric populations and the development of allergic diseases is largely undetermined. Retrieved from a longitudinal birth cohort, the first aim was to document serum EDN levels at 1 and 3 years in general, and in non-atopic children specifically, and second, to explore possible sex differences in EDN levels at both ages. The third aim was to investigate potential associations between EDN levels at 1 and 3 years and any wheeze, preschool asthma, and preschool asthma with AS and/or AD at 3 years.

2 | METHODS

2.1 | Study design

The present study addressed observational research questions based on information prospectively collected from the Preventing Atopic Dermatitis and ALLergies in children (PreventADALL) study. This Scandinavian mother–child cohort, recruiting from the general population, with two randomized controlled interventions; skin care and early food introduction, is described elsewhere.[23,24] Briefly, the mothers were recruited during the routine ultrasound examination in mid-pregnancy in Norway and Sweden, between 2014–2016. The children, born without serious illnesses at gestational age (GA) ≥35 weeks, were enrolled during the first days of life. The children were invited to clinical follow-ups at 3 and 6 months, and at 1, 2 and 3 years. Informed written consent was obtained from the mothers at enrolment, and from both parents at newborn inclusion.

2.2 | Study population

The present study population consisted of 1233/2394 children from the PreventADALL study with serum EDN levels available at 1 and/or 3 years (Figure 1).

2.3 | Data collection

2.3.1 | Serum EDN

Blood samples, collected at 1 and 3 years, were kept in room temperature for 60–90 min before centrifugation and separation of serum, thereafter kept at −20°C for 5–7 days before transferred for storage at −80°C. Serum EDN was measured using ImmunoCAP (Thermo Fisher Scientific, Uppsala, Sweden), according to protocol.[9]

2.3.2 | Preschool asthma and wheeze

Information on doctor diagnosed asthma and wheeze was collected from questionnaires (3, 6, 9, 12, 18, 24, 30 and 36 months) and interviews (24 and 36 months). Asthma medication use was retrieved from questionnaires (12, 18, 24, 30 and 36 months). To compensate for missing information in some participants, the children's medical records were reviewed for the prescription of asthma medication between 9 months through 3 years.

2.3.3 | Allergic sensitization

Skin prick tests (SPT) were performed at 3 years using standard allergen solutions for egg, cow's milk, peanut, wheat, soy, cod,

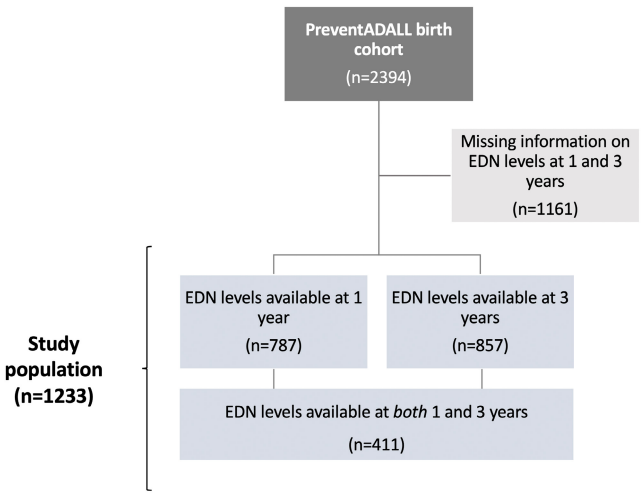


FIGURE 1 Flow chart of the study population ($n = 1233$). EDN, eosinophil-derived neurotoxin; PreventADALL, Preventing Atopic Dermatitis and ALLergies in children.

birch, grass, dog, cat and house dust mite (Soluprick ALK-Albelló, Hørsholm, Denmark). In addition, at 3 years analyses for specific serum immunoglobulin E (IgE) to egg, cow's milk, peanut, wheat, soy, cod, birch, grass, mugwort, dog, cat, horse, house dust mite and mould (*Cladosporium herbarium*) were performed using ImmunoCAP (Thermo Fisher Scientific). Allergic sensitization was defined as having a median SPT wheal diameter of ≥3 mm after 15 min subtracting the diameter of the negative control and/or a specific IgE level >0.35 kU_A/L towards at least one allergen.

2.3.4 | Clinical skin assessments

Examinations were carried out at 3 years by trained study personnel educated together at workshops to minimize inter-observer variability, using the United Kingdom Working Party (UKWP) and/or Hanifin and Rajka criteria to diagnose children with AD.[25]

2.3.5 | Birth and background characteristics

Background data on socio-demographic characteristics, lifestyle and environmental factors were gathered from electronic questionnaires at 18 and 34 weeks of pregnancy. Birth data was collected from electronic medical records.

2.4 | Definitions and outcomes

2.4.1 | Non-atopic children

Non-atopic children at 3 years were defined as children with no history of asthma, wheeze, rBO, doctor diagnosed asthma, use of and/or prescribed asthma medications, AS or AD between birth and 3 years.

2.4.2 | Primary outcome

Serum EDN levels at 1 and 3 years are presented with median, interquartile range (IQR), minimum–maximum (min–max), and the 95th percentile, due to the skewed distributions of EDN levels. The latter measure is defined as the upper limit of normal (ULN) in non-atopic children.

2.4.3 | Secondary outcomes

Any wheeze at 3 years was defined as children with any episode of wheeze between 2 and 3 years, not fulfilling the preschool asthma criteria at 3 years.

Preschool asthma at 3 years was defined as children with ≥3 episodes of recurrent bronchial obstruction (rBO) between 2 and 3 years, and fulfilling minimum one of the following criteria; (1) doctor diagnosed asthma between 0 and 3 years, and (2) any use of and/or prescribed asthma medications (bronchodilators, inhaled corticosteroids or leukotriene-antagonist) between 9 months and 3 years.[26]

Preschool asthma with AS and/or AD at 3 years was defined as having a positive SPT (≥3 mm) and/or specific IgE test (>0.35 kU_A/L) towards any allergen and/or fulfilling the diagnostic criteria for AD through either UKWP and/or Hanifin and Rajka at 3 years, in addition to meeting the criteria for preschool asthma.

2.5 | Statistical analyses

Birth and background characteristics were compared using independent *t*-tests for continuous variables presented with means and standard deviations (SD), and χ^2 tests or Fisher's exact tests for categorical variables presented with numbers (*n*) and percentages (%). The relationship between EDN levels at 1 and 3 years was examined in a robust linear regression model presented with β-coefficient with 95% confidence interval (95% CI), coefficient of determination (R^2) and correlation coefficient (*r*) with 95% CI. Similarly, among the children with paired samples at 1 and 3 years, EDN levels in general and in non-atopic children were compared with Wilcoxon's signed rank test. EDN levels at 1 and 3 years in children with any wheeze, preschool asthma, AS, AD, preschool asthma with AS and/or AS and any allergic disease at 3 years were compared to EDN levels in non-atopic children, using Mann–Whitney *U*-tests. To determine cut-off levels for 'higher EDN levels' at 1 and 3 years for any wheeze, preschool asthma and preschool asthma with AS and/or AD at 3 years, Youden Index[27] were estimated and EDN levels accordingly dichotomized. Among the atopic and non-atopic children, associations between continuous and dichotomized EDN cut-off levels at 1 and 3 years and any wheeze, preschool asthma and preschool asthma with AS and AD at 3 years were investigated in univariate and multivariate logistic regression models, presented with odds ratio (OR) with 95% CI. Further details are described in Data S1.

3 | RESULTS

The study population consisted of 1233 children of whom 573 (46.5%) were girls, with a mean (SD) age of 12.5 (0.73) and 38.0 (3.65) months at clinical follow-ups (Table 1). Birth and background characteristics among the 411 children with EDN levels available at both 1 and 3 years are described in Table S1. At 3 years, 151/810 (18.6%) had reported any wheeze while not fulfilling the preschool asthma criteria, 109/892 (12.2%) were defined with preschool asthma, 189/1090 (17.3%) were sensitized to at least one allergen, and 269/1108 (22.6%) were diagnosed with AD. In S1 A–B, the distribution of allergic diseases as well as non-atopic children are illustrated, respectively. Birth and background characteristics of non-atopic children and children with preschool asthma at 3 years are described in Table S2.

TABLE 1 Birth and background characteristics of the included children with information on EDN at 1 and/or 3 years ($n = 1233$) compared with the remaining cohort without information on EDN at 1 and/or 3 years ($n = 1161$).

	Children with EDN at 1 and/or 3 years ($n = 1233$)		Remaining cohort ($n = 1161$)		
Characteristics	***n* (%) or mean (SD; min–max)**	**No.**	***n* (%) or mean (SD; min–max)**	**No.**	***p*-value[a]**
Parents					
Nordic origin mother	1042 (90.9)	1146	921 (89.9)	1025	.397
Nordic origin father	1024 (91.3)	1122	879 (88.2)	997	.019
High household education ≥4 years	795 (71.2)	1117	660 (66.0)	1000	.010
Urban living environment pregnancy	1084 (94.6)	1146	929 (90.6)	1025	<.001
Married/cohabitant mother	1123 (97.7)	1149	1000 (97.1)	1030	.339
Parity ≥1	399 (40.5)	1231	463 (39.9)	1160	.756
Tobacco smoke in pregnancy	52 (4.20)	1233	52 (4.50)	1161	.754
Any parental allergic disease	721 (64.0)	1126	639 (64.4)	992	.854
Parental asthma	311 (28.8)	1081	296 (31.3)	947	.222
Parental atopic dermatitis	334 (31.4)	1063	281 (31.2)	930	.561
Parental food allergy	248 (23.7)	1046	203 (22.6)	900	.548
Parental allergic rhinitis	443 (42.5)	1043	388 (43.3)	896	.713
Infancy (0–1 year)					
Girl	573 (46.5)	1233	566 (48.8)	1161	.264
GA in weeks	40.1 (1.32; 35.3–42.4)	1228	40.0 (1.38; 35.0–42.6)	1160	.181
Caesarean section	187 (15.2)	1231	210 (18.1)	1159	.055
Birth weight in kg	3.56 (0.47; 2.00–4.96)	1227	3.58 (0.49; 1.79–5.63)	1157	.314
Birth length in cm	50.5 (2.06; 42.0–61.0)	1180	50.6 (2.09; 41.0–58.0)	1081	.300
Age in months at 1-year follow-up	12.5 (0.73; 10.8–15.4)	1189	12.6 (0.79; 10.4–15.8)	716	.161
Eosinophil-derived neurotoxin (EDN)					
Information at 1 year	787 (63.8)	1233	–	–	na
Information at 3 years	857 (69.5)	1233	–	–	na
Information at both ages	411 (33.3)	1233	–	–	na
Children (3 years)					
Age in months	38.0 (3.65; 33.0–62.0)	1199	40.0 (6.04; 34.0–62.0)	735	<.001
Weight in kg	15.2 (1.72; 10.5–21.6)	1113	15.3 (1.67; 11.3–21.1)	515	.453
Any wheeze[b]	151 (18.6)	810	88 (20.9)	421	.341
Preschool asthma[c]	109 (12.2)	892	73 (16.0)	457	.056
Allergic sensitization[d]	189 (17.3)	1090	36 (8.2)	439	<.001
Atopic dermatitis[e]	269 (22.6)	1108	101 (19.1)	529	.019
Any allergic disease[f]	338 (38.9)	869	19 (7.20)	264	<.001

Abbreviations: EDN, eosinophil-derived neurotoxin; GA, gestational age; IgE, immunoglobulin E.; na, not applicable; No., number; SPT, skin prick test.

[a]Independent *t*-test or χ^2 test.

[b]Any wheeze at 3 years was defined as children with any episode of wheeze between 2 and 3 years, not fulfilling the preschool asthma criteria at 3 years.

[c]Preschool asthma at 3 years was defined as having ≥3 episodes of recurrent bronchial obstruction (rBO) between 2 and 3 years, and fulfilling minimum one of the following criteria; (1) doctor diagnosed asthma between 0 and 3 years, and (2) any use of and/or prescribed asthma medications (bronchodilators, inhaled corticosteroids or leukotriene-antagonist) between 9 months and 3 years.

[d]Allergic sensitization at 3 years was defined as having positive SPT (≥3 mm) and/or specific IgE level (>0.35 kU_A/L) towards any allergen (egg, cow's milk, peanut, wheat, soy, cod, birch, grass, mugwort, dog, cat, horse, house dust mite and/or mould) at 3 years.

[e]Atopic dermatitis at 3 years was defined as fulfilling the United Kingdom Working Party and/or Hanifin and Rajka criteria at 3 years.

[f]Any allergic disease at 3 years was defined as children with either any wheeze, preschool asthma, allergic sensitization and/or atopic dermatitis at 3 years.

3.1 | EDN levels in infancy and at preschool age

Levels of EDN were available in 787/1233 (63.8%) children at 1 year and in 857/1233 (69.5%) children at 3 years, while 411/1233 (33.3%) children had information available at both ages. The median EDN level (μg/L) in all children was 27.4 at 1 year, and 20.1 at 3 years, with the corresponding EDN levels in non-atopic children of 24.0 and 17.3. In the children with EDN levels at both ages, the median EDN levels were higher at 1 compared to at 3 years; 27.8 versus 19.9 ($p < .001$) (Figure 2B, Table 2) as well as in the 93 non-atopic children; 23.7 versus 15.9 ($p < .001$) (Table 2).

EDN levels at 1 year positively correlated to EDN levels at 3 years ($n = 411$), β-coefficient (95% CI) 0.62 (0.44, 0.81), $R^2 = 0.23$, ($p < .001$) and r (95% CI) 0.49 (0.40, 0.55) ($p < .001$), see Figure 3.

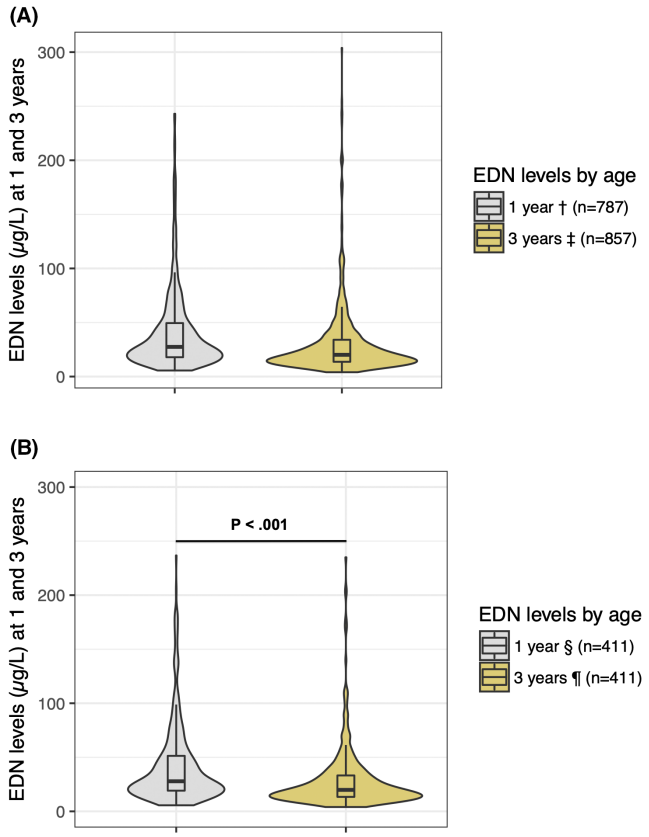


FIGURE 2 EDN levels at 1 ($n = 787$) and 3 (A) ($n = 857$) years, and in children with information on EDN at both ages (B) ($n = 411$). From the bottom of the boxes, horizontal lines represent the second quartile, median and third quartile. Vertical lines below and above are the first and fourth quartile, respectively. The narrow tips represent EDN levels above the fourth quartile. † EDN levels at 1 year among all children with information on EDN at 1 year. ‡ EDN levels at 3 years among all children with information on EDN at 3 years. § EDN levels at 1 year among children with information at both 1 and 3 years. ¶ EDN levels at 3 years among children with information at both 1 and 3 years. EDN, eosinophil-derived neurotoxin; max, maximum; μg/L, micrograms per litre; min, minimum.

3.1.1 | EDN levels by sex

In all children, the median EDN levels (μg/L) were higher in boys compared to girls; 32.0 versus 24.5 at 1 year and 20.9 versus 19.0 at 3 years ($p < .001$), whereas in non-atopic children, the levels at 1 and 3 years were similar between the sexes (Table 3). In the 411 children with EDN levels available at both ages, higher median EDN levels were observed in boys ($n = 239$) compared to girls ($n = 172$); 31.3 versus 24.8 at 1 year and 20.2 versus 18.5 at 3 years (p-values $< .001$), whereas no differences in EDN levels were observed between boys ($n = 47$) and girls ($n = 46$) in the 93 non-atopic children; 24.0 versus 23.3 at 1 year, and 15.5 and 16.4 at 3 years (p-values $> .05$), respectively.

3.1.2 | EDN levels in children with preschool asthma and other allergic diseases

Compared to non-atopic children, the median EDN levels at 1 year were higher in children with any wheeze ($p = .001$), preschool asthma ($p = .025$), AS ($p < .001$), and AD ($p < .001$) (Figure 4A), as well as preschool asthma with AS and/or AD ($p = .002$), and any allergic disease ($p < .001$) at 3 years (Table 2). Compared to non-atopic children, the median EDN levels at 3 years were higher in children with any wheeze ($p = .016$), preschool asthma ($p < .001$), AS ($p < .001$) and AD ($p = .001$) (Figure 4B), as well as preschool asthma with AS and/or AD ($P < .001$), and any allergic disease ($p < .001$) at 3 years (Table 2). Compared to girls, boys with preschool asthma, AD and any allergic disease at 3 years had higher median EDN levels at 1 year, while median EDN levels at 3 years were higher in boys with any wheeze at 3 years (p-values $< .05$; Table 3).

At 1 year, EDN levels above the ULN of non-atopic children (>107 μg/L) were observed in 7/94 (7.4%) with any wheeze, 8/68 (11.8%) with preschool asthma, 13/95 (13.7%) with AS, 13/154 (8.4%) with AD, 4/31 (12.9%) preschool asthma with AS and/or AD, and 27/311 (8.7%) and any allergic disease at 3 years. The corresponding rates at 3 years (EDN levels >84.6 μg/L) were 9/107 (8.4%), 4/76 (5.3%), 20/176 (11.4%), 14/201 (7.0%), 3/38 (7.9%) and 28/404 (6.9%) of the children with any wheeze, preschool asthma, AS, AD, preschool asthma with AS and/or AD, and any allergic disease at 3 years, respectively.

3.1.3 | EDN levels in relation to preschool asthma and other allergic diseases

Among all children, higher EDN levels (μg/L) were identified at 1 and 3 years for any wheeze as ≥27.8 and ≥20.6, respectively, for preschool asthma ≥26.7 and ≥20.5, respectively, and for preschool asthma with AS and/or AD ≥27.8 and ≥20.6 at 3 years, respectively. Higher EDN levels at 1 year were associated with any wheeze, preschool asthma and preschool asthma with AS and/or AD at

TABLE 2 EDN levels in children with information at 1 ($n=787$) and 3 ($n=857$) years, at both ages ($n=411$), as well as EDN levels at 1 and 3 years by atopic status at 3 years.

	1 year			3 years			
Characteristics	Median (IQR; min–max)	95th percentile	No.	Median (IQR; min–max)	95th percentile	No.	*P*-value[a]
EDN levels (μg/L) by age in general							
1 year	27.4 (31.7; 5.61–243)	121	787				na
3 years				20.1 (20.4; 4.00–304)	87.8	857	na
Both 1 and 3 years	27.8 (32.3; 5.61–237)	134	411	19.9 (20.1; 4.00–235)	80.0	411	<.001
EDN levels (μg/L) in non-atopic children[c]							
1 year	24.0 (31.1; 5.86–216)	107[b]	147				na
3 years				17.3 (17.0; 5.00–245)	84.6[b]	173	na
Both 1 and 3 years	23.7 (34.4; 5.86–184)	114	93	15.9 (17.9; 6.28–109)	71.1	93	<.001
EDN levels (μg/L) by allergic disease							
Any wheeze at 3 years[d]	37.3 (33.3; 6.40–212)	142	94	20.9 (22.0; 4.00–254)	174	107	na
Preschool asthma at 3 years[e]	32.6 (44.1; 6.14–237)	163	68	25.1 (20.5; 7.10–304)	89.9	76	na
Allergic sensitization at 3 years[f]	42.0 (46.9; 9.10–205)	168	95	28.3 (30.5; 7.15–304)	136	176	na
Positive SPT at 3 years	42.2 (43.8; 18.3–205)	160	48	28.3 (32.4; 8.18–254)	184	75	na
Positive specific IgE test at 3 years	42.1 (57.4; 9.10–205)	175	78	29.8 (31.3; 7.15–304)	138	168	na
Atopic dermatitis at 3 years[g]	35.5 (43.2; 5.85–242)	139	154	22.5 (21.9; 5.72–254)	113	201	na
Preschool asthma with AD and/or AS at 3 years[h]	42.8 (42.4; 8.24–174)	167	31	28.0 (28.8; 7.10–304)	238	38	na
Any allergic disease at 3 years[i]	33.5 (38.3; 5.85–242)	146	311	22.6 (23.0; 4.00–304)	107	404	na

Abbreviations: EDN, eosinophil-derived neurotoxin; IgE, immunoglobulin E; na, not applicable; no., number; SPT, skin prick test; μg/L, micrograms per litre.

[a]Wilcoxon's signed rank test.

[b]Upper limit of normal (ULN) was defined as the 95th percentile.

[c]Non-atopic children were defined as children with no history of asthma, wheeze, rBO episodes, doctor diagnosed asthma, use of and/or prescribed asthma medications, atopic dermatitis or allergic sensitization between birth and 3 years.

[d]Any wheeze at 3 years was defined as children with any episode of wheeze between 2 and 3 years, not fulfilling the preschool asthma criteria at 3 years.

[e]Preschool asthma at 3 years was defined as having ≥3 episodes of recurrent bronchial obstruction (rBO) between 2 and 3 years, and fulfilling minimum one of the following criteria; (1) doctor diagnosed asthma between 0 and 3 years, and (2) any use of and/or prescribed asthma medications (bronchodilators, inhaled corticosteroids or leukotriene-antagonist) between 9 months and 3 years.

[f]Allergic sensitization at 3 years was defined as having positive SPT (≥3 mm) and/or specific IgE level (>0.35 kU_A/L) towards any allergen (egg, cow's milk, peanut, wheat, soy, cod, birch, grass, mugwort, dog, cat, horse, house dust mite and/or mould) at 3 years.

[g]Atopic dermatitis at 3 years was defined as fulfilling the United Kingdom Working Party and/or Hanifin and Rajka criteria at 3 years.

[h]Preschool asthma with AS and/or AD at 3 years was defined as having allergic sensitization and/or atopic dermatitis at 3 years, in addition to meeting the criteria for preschool asthma at 3 years.

[i]Any allergic disease at 3 years was defined as children with either any wheeze, preschool asthma, allergic sensitization and/or atopic dermatitis at 3 years.

3 years; adjusted OR (95% CI) 2.80 (1.49, 5.28), 2.20 (1.09, 4.41) and 4.61 (1.60, 13.3), respectively (Figure S2A, Table 4). Higher EDN levels at 3 years were associated with all three clinical outcomes at 3 years, see Table 4. Including paired EDN levels (1 and 3 years) in the analyses, only the associations between EDN levels at 1 year for any wheeze as well as EDN levels at 3 years for preschool asthma at 3 years were significant (Table S3). The linear relationship between continuous EDN levels at 1 and 3 years by atopic status at 3 years is shown in Figure S2.

Higher EDN levels at 1 year for differentiating preschool asthma at 3 years from non-atopic children had a sensitivity of 61.8%, specificity of 57.1% and a ROC-AUC (95% CI) of 0.60 (0.51, 0.68). The predictive accuracy of EDN levels for differentiating children with any wheeze, preschool asthma and preschool asthma with AS and/or AD at 3 years from non-atopic children are described in Figure S3 and Table S4.

4 | DISCUSSION

In this study of 1233 non-selected children, we report serum EDN levels at 1 and 3 years. Levels of EDN were higher at 1 compared to 3 years, also observed in non-atopic children, and in boys compared to girls at both ages. Higher EDN levels at age 1 and 3 years were

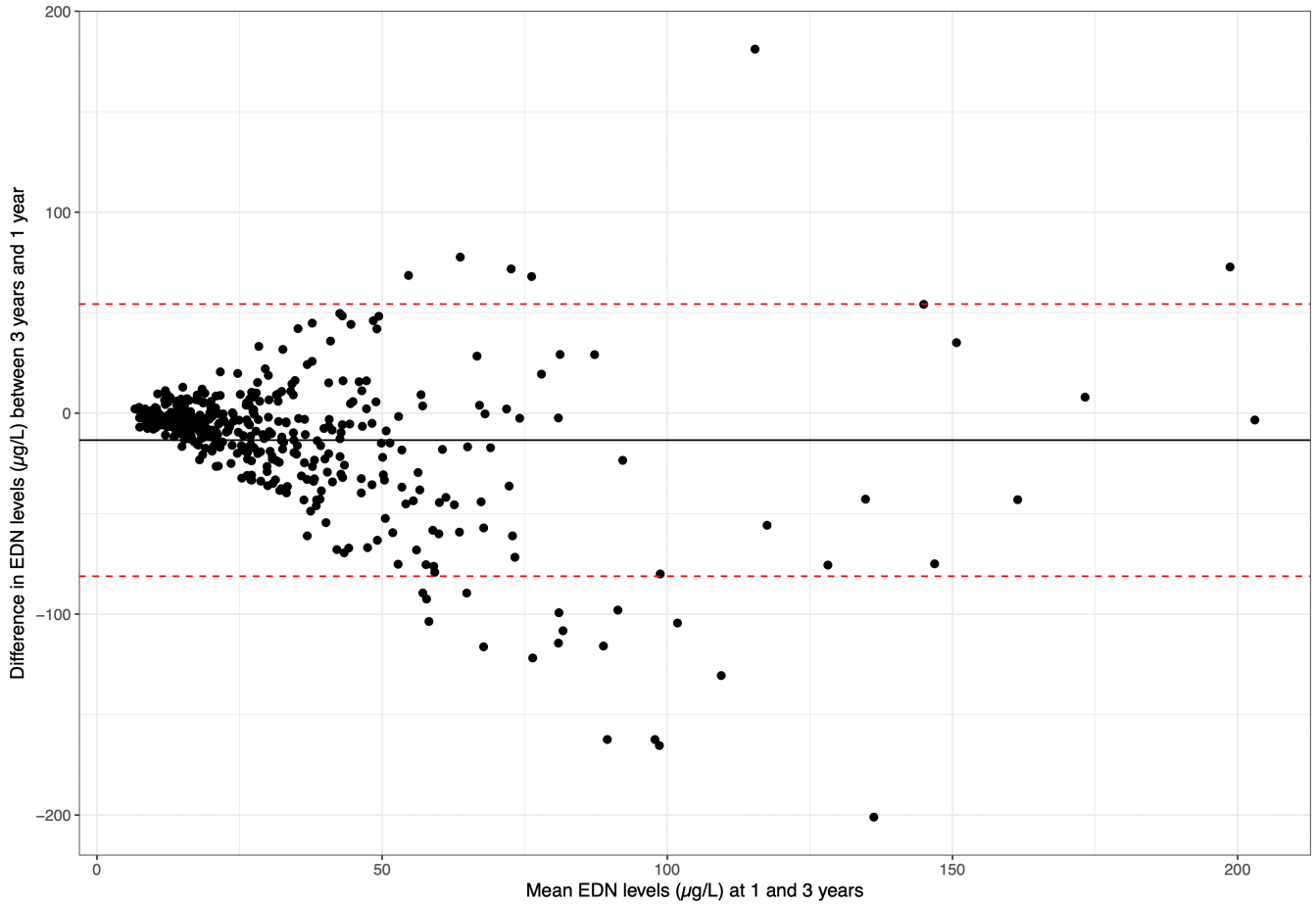


FIGURE 3 Bland–Altman plot, displaying the difference of the paired EDN measurements at 1 and 3 years plotted against the mean of the two measurements ($n = 411$). The red dashed lines in the plot represents ± 2 SD. EDN, eosinophil-derived neurotoxin; µg/L, micrograms per litre.

associated with any wheeze, preschool asthma and preschool asthma with AS and/or AD at 3 years.

To our knowledge, this is the first report describing EDN levels in non-selected and in non-atopic children retrieved from a large paediatric population at two distinctive time points in early childhood. Our finding of higher levels at 1 year compared to that at 3 years is supported by similar observations for eosinophil cationic protein (ECP) levels, with higher levels in infancy (0–23 months) compared to that at preschool age (24–41 months), among 245 children.[28] On the other hand, two smaller studies found no age differences in ECP levels over a larger age spectrum in childhood (0–15 and 0–12 years, respectively).[29,30] Also, blood eosinophil counts were higher in infancy compared to that at preschool- and school age, and in younger children compared to adolescents, in two large studies establishing reference values.[31,32] Thus, a higher blood eosinophil count in infancy might hypothetically explain our novel observations.

We are unaware of other reports showing higher EDN levels in boys compared to girls at 1 and 3 years. In line with our findings, in adults, Granger et al. reported higher EDN levels in males compared to females.[8] In support, male sex has also been associated with higher eosinophil counts in children.[33] Of note, the sex differences were no longer apparent among non-atopic children, perhaps suggesting that more frequent allergic disease in boys may account for the higher levels observed in boys.

The higher EDN levels at both 1 and 3 years in children with any wheeze, preschool asthma, AS, AD, preschool asthma with AS and/or AD, and any allergic disease compared to non-atopic children at 3 years, are in line with several case–control studies.[9,10,12–18,34,35] In Rydell et al.'s study of 213 web-recruited children,[9] median EDN levels were higher in children with concurrent asthma (74.1 µg/L) compared to the children with preschool asthma in our study (25.1 µg/L). Differences in age, populations and asthma definitions may explain these discrepancies. Several novel findings were observed in relation to EDN at 1 year and allergic diseases by 3 years of age; higher EDN levels at 1 year in children who at 3 years had asthma-like symptoms (any wheeze and preschool asthma), as well as children with AS and AD, compared to children who remained non-atopic by 3 years. Interestingly, sensitized children had among the highest EDN levels observed at both 1 and 3 years, pointing to the involvement of eosinophil inflammation. In relation to children with preschool asthma, children with preschool asthma and comorbidities of AS and/or AD had among the highest EDN levels at 1 year, which largely may be driven by sensitization, indicating a phenotype in which eosinophil inflammation may be established

TABLE 3 EDN levels by sex in children with information at 1 ($n=787$) and 3 ($n=857$) years, as well as by atopic status at 3 years.

	Girls			Boys			
Characteristics	Median (IQR; min–max)	95th percentile	No.	Median (IQR; min–max)	95th percentile	No.	*p*-value[a]
EDN levels (μg/L) by age in general							
1 year	24.5 (28.1; 5.61–242)	97.0	363	32.0 (34.9; 5.95–243)	133	424	<.001
3 years	19.0 (18.4; 4.00–235)	72.4	382	20.9 (21.9; 5.05–304)	96.3	475	.018
EDN levels (μg/L) in non-atopic children[c]							
1 year	21.4 (30.4; 5.86–186)	86.5[b]	82	25.4 (31.2; 5.95–216)	129[b]	65	.212
3 years	16.8 (16.0; 5.00–105)	65.3[b]	85	17.4 (17.5; 6.30–245)	107[b]	88	.988
EDN levels (μg/L) at 1 year by allergic disease							
Any wheeze at 3 years[d]	36.3 (39.0; 11.0–205)	169	39	38.0 (31.0; 6.40–212)	143	55	.487
Preschool asthma at 3 years[e]	24.8 (34.4; 6.14–175)	170	26	36.5 (44.8; 8.60–237)	160	42	.032
Allergic sensitization at 3 years[f]	34.6 (48.2; 11.7–205)	181	35	43.8 (42.1; 9.10–183)	165	60	.203
Positive SPT at 3 years	34.5 (30.5; 18.3–205)	167	18	45.4 (39.9; 20.4–156)	147	30	.187
Positive specific IgE at 3 years	35.0 (68.9; 11.7–205)	194	26	42.5 (43.2; 9.10–183)	171	52	.539
Atopic dermatitis at 3 years[g]	26.6 (27.5; 5.85–242)	130	69	42.2 (52.6; 9.83–183)	140	85	.003
Preschool asthma with AD and/or AS at 3 years[h]	36.2 (39.0; 8.24–175)	173	11	44.3 (45.7; 9.10–121)	121	20	.887
Any allergic disease at 3 years[i]	25.9 (30.0; 5.85–242)	154	139	37.7 (42.6; 6.40–237)	146	172	<.001
EDN levels (μg/L) at 3 years by allergic disease							
Any wheeze at 3 years[d]	15.7 (13.0; 4.00–201)	161	50	28.1 (30.2; 7.61–254)	209	57	<.001
Preschool asthma at 3 years[e]	24.4 (41.8; 11.8–235)	203	23	27.5 (20.5; 7.10–304)	92.1	53	.560
Allergic sensitization at 3 years[f]	25.7 (30.2; 7.68–235)	114	67	29.5 (31.4; 7.15–304)	154	109	.262
Positive SPT at 3 years	25.6 (34.0; 8.18–235)	218	29	33.5 (33.9; 10.7–254)	157	46	.227
Positive specific IgE at 3 years	29.2 (31.1; 7.68–235)	117	62	29.8 (32.5; 7.15–304)	158	106	.396
Atopic dermatitis at 3 years[g]	22.5 (21.9; 6.67–235)	114	87	22.8 (22.6; 5.72–254)	120	114	.552
Preschool asthma with AD and/or AS at 3 years[h]	32.3 (44.4; 16.6–235)	219	10	25.9 (20.4; 7.10–304)	207	28	.272
Any allergic disease at 3 years[i]	21.4 (21.8; 4.00–235)	102	168	23.7 (24.4; 5.72–304)	111	236	.075

Abbreviations: EDN, eosinophil-derived neurotoxin; IgE, immunoglobulin E; na, not applicable; no., number; SPT, skin prick test; μg/L, micrograms per litre.

[a]Mann–Whitney *U*-test.

[b]Upper limit of normal (ULN) was defined as the 95th percentile.

[c]Non-atopic children were defined as children with no history of asthma, wheeze, rBO episodes, doctor diagnosed asthma, use of and/or prescribed asthma medications, atopic dermatitis or allergic sensitization between birth and 3 years.

[d]Any wheeze at 3 years was defined as children with any episode of wheeze between 2 and 3 years, not fulfilling the preschool asthma criteria at 3 years.

[e]Preschool asthma at 3 years was defined as having ≥3 episodes of recurrent bronchial obstruction (rBO) between 2 and 3 years, and fulfilling minimum one of the following criteria; (1) doctor diagnosed asthma between 0 and 3 years, and (2) any use of and/or prescribed asthma medications (bronchodilators, inhaled corticosteroids or leukotriene-antagonist) between 9 months and 3 years.

[f]Allergic sensitization at 3 years was defined as having positive SPT (≥3 mm) and/or specific IgE level (>0.35 kU_A/L) towards any allergen (egg, cow's milk, peanut, wheat, soy, cod, birch, grass, mugwort, dog, cat, horse, house dust mite and/or mould) at 3 years.

[g]Atopic dermatitis at 3 years was defined as fulfilling the United Kingdom Working Party and/or Hanifin and Rajka criteria at 3 years.

[h]Preschool asthma with AS and/or AD at 3 years was defined as having allergic sensitization and/or atopic dermatitis at 3 years, in addition to meeting the criteria for preschool asthma at 3 years.

[i]Any allergic disease at 3 years was defined as children with either any wheeze, preschool asthma, allergic sensitization and/or atopic dermatitis at 3 years.

already in infancy, in line with EDN being associated with AS, allergic rhinitis and AD in school children.[16] Similarly, in the meta-analysis by Bao et al., in relation to tobacco smoke exposure, sex, heredity for asthma and wheezing, children with AS and AD had the highest likelihood of developing asthma by preschool- or school age.[36] However, EDN levels were overall somewhat higher in children with any wheeze or preschool asthma compared to non-atopic children, suggesting eosinophil inflammation may also be involved in children with early asthma-like symptoms in the absence of AS and/or AD.

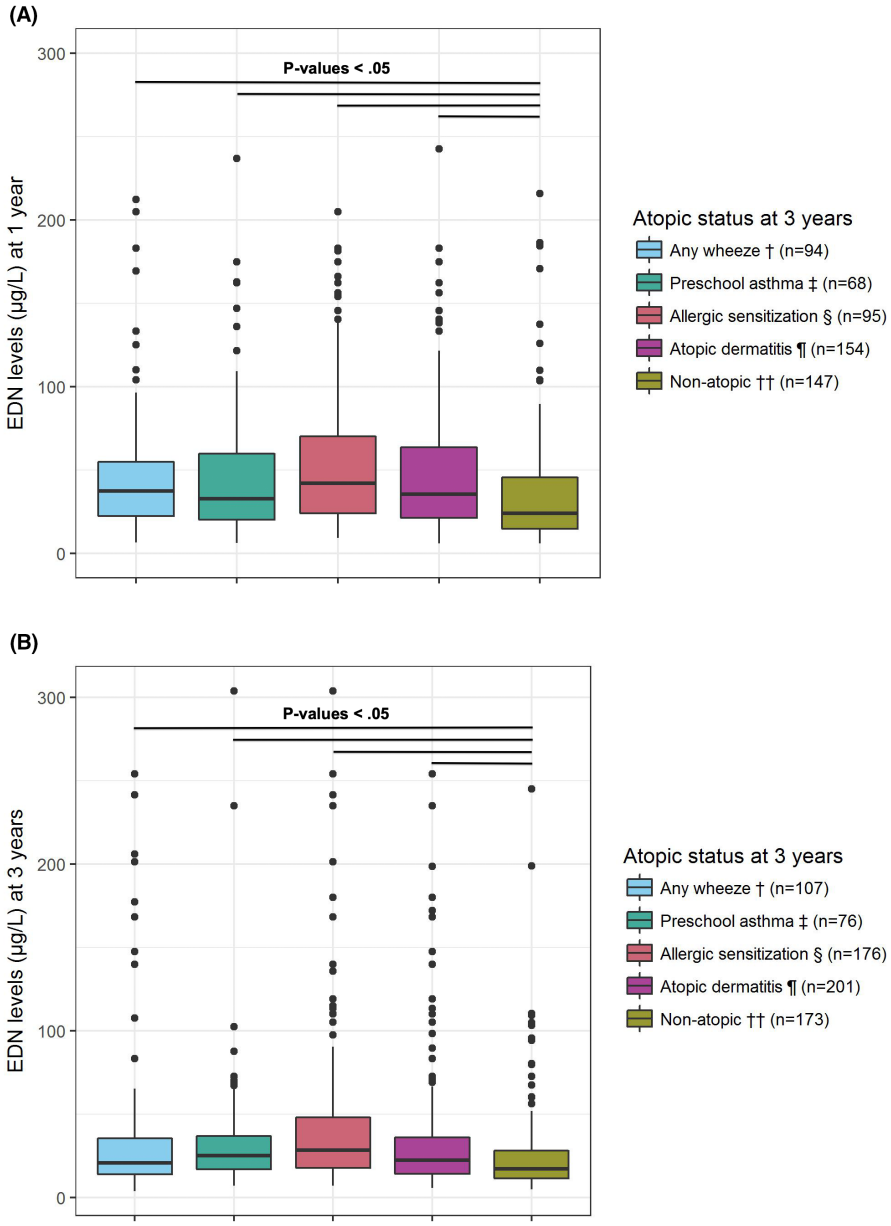


FIGURE 4 EDN levels at 1 (A) and 3 (B) years by atopic status at 3 years. From the bottom of the boxes, horizontal lines represent the second quartile, median and third quartile. Vertical lines below and above are the first and fourth quartile, respectively. The dots represent EDN levels above the fourth quartile. † Any wheeze at 3 years was defined as children with any episode of wheeze between 2 and 3 years, not fulfilling the preschool asthma criteria at 3 years. ‡ Preschool asthma at 3 years was defined as having ≥3 episodes of recurrent bronchial obstruction (rBO) between 2 and 3 years, and fulfilling minimum one of the following criteria; (1) doctor diagnosed asthma between 0 and 3 years, and (2) any use of and/or prescribed asthma medications (bronchodilators, inhaled corticosteroids or leukotriene-antagonist) between 9 months and 3 years. § Allergic sensitization at 3 years was defined as having positive SPT (≥3 mm) and/or specific IgE level (>0.35 kU_A/L) towards any allergen (egg, cow's milk, peanut, wheat, soy, cod, birch, grass, mugwort, dog, cat, horse, house dust mite and/or mould) at 3 years. ¶ Atopic dermatitis at 3 years was defined as fulfilling the United Kingdom Working Party and/or Hanifin and Rajka criteria at 3 years. †† Non-atopic children were defined as children with no history of asthma, wheeze, rBO episodes, doctor diagnosed asthma, use of and/or prescribed asthma medications, atopic dermatitis or allergic sensitization between birth and 3 years. EDN, eosinophil-derived neurotoxin; IgE, immunoglobulin E; max, maximum; min, minimum; SPT, skin prick test; µg/L, micrograms per litre.

Higher EDN levels at 1 and 3 years were associated with any wheeze, preschool asthma and preschool asthma with AS and/or AD at 3 years, in line with previous reports on wheeze[22] and different asthma phenotypes.[16] To our knowledge, our study is the largest to investigate associations between infant EDN levels and preschool asthma, as well as EDN levels and concurrent asthma at preschool age.

4.1 | Strengths and limitations

The automated ImmunoCAP assay[9] may be beneficial over manual enzyme-linked immunosorbent assays.[37] The distinct definitions of any wheeze, preschool asthma, AS, AD, preschool asthma with AS and/or AD, and any allergic disease is strength.

TABLE 4 Crude and adjusted logistic regression models of EDN at 1 and 3 years and any wheeze, preschool asthma, and preschool asthma with allergic sensitization and/or atopic dermatitis at 3 years.

	Any wheeze at 3 years[a]				Preschool asthma at 3 years[b]				Preschool asthma with AS and/or AS at 3 years[c]			
Characteristics	**Crude OR (95% CI)**	***p*-value**	**Adjusted OR (95% CI)**	***p*-value[d]**	**Crude OR (95% CI)**	***p*-value**	**Adjusted OR (95% CI)**	***p*-value[d]**	**Crude OR (95% CI)**	***p*-value**	**Adjusted OR (95% CI)**	***p*-value[d]**
EDN at 1 year												
Continuous EDN	1.01 (1.00, 1.01)	.054	1.01 (1.00, 1.02)	.084	1.01 (1.00, 1.01)	.049	1.01 (1.00, 1.02)	.075	1.01 (1.00, 1.02)	.026	1.01 (1.00, 1.02)	.119
Higher EDN levels[e]	2.89 (1.69, 4.96)	<.001	2.80 (1.49, 5.28)	.001	2.15 (1.20, 3.88)	<.001	2.20 (1.09, 4.41)	.027	4.29 (1.80, 10.2)	<.001	4.61 (1.60, 13.3)	.005
EDN at 3 years												
Continuous EDN	1.01 (1.00, 1.01)	.025	1.01 (1.00, 1.02)	.021	1.01 (1.00, 1.02)	.033	1.02 (1.01, 1.03)	.006	1.01 (1.01, 1.02)	.014	1.02 (1.01, 1.04)	.005
Higher EDN levels[e]	1.69 (1.04, 2.76)	.035	1.95 (1.11, 3.44)	.021	2.94 (1.68, 5.16)	<.001	4.68 (2.29, 9.55)	<.001	4.65 (2.12, 10.2)	<.001	11.4 (3.57, 36.3)	<.001

Abbreviations: AD, atopic dermatitis; AS, allergic sensitization; EDN, eosinophil-derived neurotoxin; GA, gestational age; IgE, immunoglobulin E; SPT, skin prick test; μg/L, micrograms per litre.

[a]Any wheeze at 3 years was defined as children with any episode of wheeze between 2 and 3 years, not fulfilling the preschool asthma criteria at 3 years.

[b]Preschool asthma at 3 years was defined as having ≥3 episodes of recurrent bronchial obstruction (rBO) between 2 and 3 years, and fulfilling minimum one of the following criteria; (1) doctor diagnosed asthma between 0 and 3 years, and (2) any use of and/or prescribed asthma medications (bronchodilators, inhaled corticosteroids or leukotriene-antagonist) between 9 months and 3 years.

[c]Preschool asthma with AS and/or AD at 3 years was defined as having a positive SPT (≥3 mm) and/or specific IgE (>0.35 kU_A/L) towards any allergen (egg, cow's milk, peanut, wheat, soy, cod, birch, grass, mugwort, dog, cat, horse, house dust mite and/or mould) and/or fulfilling the diagnostic criteria for AD through either United Kingdom Working Party and/or Hanifin and Rajka at 3 years, in addition to meeting the criteria for preschool asthma at 3 years.

[d]Logistic regression models adjusted for tobacco smoke in pregnancy, parental allergic disease (any), sex, GA at birth, caesarean section and the PreventADALL interventions.

[e]Cut-off levels for higher EDN levels by Youden's Index for any wheeze, preschool asthma and preschool asthma with AS and/or AD at 3 years equal to EDN levels (μg/L) ≥ 27.8, ≥26.7 and ≥ 27.8 at 1 year, and ≥ 20.6, ≥20.5 and ≥ 20.6 at 3 years, respectively.

With the objective to document EDN levels, the slightly higher education level and proportion of allergic diseases among the parents in the PreventADALL cohort than in the general population[24] limit the generalizability of our observations to similar populations, as disease burden,[38] and thereby likely EDN levels, may differ with socioeconomic status. Children sensitized to allergens such as tree nuts will not have been identified at 3 years, but the potential impact on observed associations is likely to be small. Nor can we account for release of EDN triggered by infections.[5] As blood eosinophil counts are not available, we are unable to determine the relative usefulness of EDN versus eosinophil counts, to expand on previous findings of associations between eosinophil count and EDN,[8–10] age[31,32] or male sex.[33] As EDN levels appear to correlate with eosinophil counts,[8–10] one may speculate that the declining trend of blood eosinophils from infancy to school age and adolescence also will be observed in EDN levels if monitored beyond preschool age.[31,32] However, the burden of allergic diseases increases with age, suggesting EDN levels may increase, in line with the higher levels in older children reported by Rydell et al.[9]

4.2 | Clinical implications for future research

Based on a large paediatric population, we document EDN levels at 1 and 3 years representing expected levels in young boys and girls, which may be considered as reference values in similar populations. Future studies in older children are however necessary to establish clinical implications of potential sex differences in eosinophil activation. Though higher EDN levels were observed across children with any wheeze, preschool asthma, AS and AD, their levels largely overlapped between the levels of non-atopic children. Furthermore, the low sensitivity and specificity of EDN levels at 1 year to predict the three clinical outcomes, indicate limited diagnostic ability of EDN, in line with our observations that approximately one of 10 with allergic disease had EDN levels above the ULN. No reliable biomarker for paediatric allergic diseases has yet been identified, suggesting the need of a comprehensive approach using a combination of diagnostic markers together with clinical observations and medical history.[39] A limited number of biomarkers for a Th2-driven inflammation are available, of which all face different challenges.[40,41] However, as an easily obtained and stable marker of eosinophil inflammation, EDN may by itself or in combination with other biomarkers be a useful complement in clinical practice.

5 | CONCLUSION

This study documented serum EDN levels, showing higher EDN levels in general at 1 year compared to at 3 years, also observed in non-atopic children, and in boys compared to girls. Compared to non-atopic children, higher EDN levels were observed at both ages in children with any wheeze, preschool asthma and preschool asthma with AS and/or AD at 3 years. Higher EDN levels at 1 and 3 years were associated with all three clinical outcomes at 3 years, but had limited diagnostic potential.

AUTHOR CONTRIBUTIONS

Martin Färdig contributed to conception and design of the study, data collection, data curation, analysis and interpretation of data, manuscript writing and editing. Anine Lie contributed to data collection, data curation and critically revised the manuscript. Magnus P. Borres contributed to conception of the study, and critically revised the manuscript. Tina Ekenkrantz contributed to data curation, and critically revised the manuscript. Berit Granum contributed to interpretation of data, and critically revised the manuscript. Guttorm Haugen contributed to interpretation of data, and critically revised the manuscript. Christine M. Jonassen contributed to interpretation of data, and critically revised the manuscript. Robert Movérare contributed to conception of the study, and critically revised the manuscript. Eva Maria Rehbinder contributed to data collection, interpretation of data and critically revised the manuscript. Håvard O. Skjerven contributed to conception and design of the study, interpretation of data and critically revised the manuscript. Anne Cathrine Staff contributed to conception and design of the PreventADALL study, interpretation of data and critically revised the manuscript. Riyas Vettukattil contributed to data curation, and critically revised the manuscript. Karin C. Lødrup Carlsen contributed to conception and design of the study, interpretation of data, and critically revised the manuscript. Cilla Söderhäll contributed to conception and design of the study, interpretation of data and critically revised the manuscript. Björn Nordlund contributed to conception and design of the study, interpretation of data, and critically revised the manuscript. All listed authors approved the final version of the manuscript before submission and agreed to be accountable for all aspects of the work.

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CONFLICT OF INTEREST STATEMENT

Thermo Fisher Scientific, Uppsala, Sweden, supported PreventADALL with the EDN analyses. Magnus P. Borres, Tina Ekenkrantz and Robert Movérare are employed by Thermo Fischer Scientific. Cilla Söderhäll has received non-financial support from Thermo Fisher Scientific in other research projects. Eva Maria Rehbinder has received honoraria for lectures from Leo Pharma, Sanofi Genzyme, Abbvie, Novartis, Norwegian Asthma and Allergy Association and Norwegian Psoriasis and Eczema Association. All other authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Participants of this study were not asked to consent for open access data from third parties.

ETHICS STATEMENT

The study was conducted according to the guidelines of the Declaration of Helsinki, and was approved by the Regional Committee for Medical and Health Research Ethics in Norway (2014/518) on December 8, 2014, and the Swedish Ethical Review Authority (2014/2242–31/4) in Sweden on March 25, 2015, registered at https://www.clinicaltrials.gov, NCT02449850 (https://www.clinicaltrials.gov/ct2/show/NCT02449850?term=PreventADALL&draw=2&rank=1, accessed on 28 November 2022). Informed written consent was collected from all mothers at enrolment, and from parent(s) at infant inclusion.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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