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ORIGINAL RESEARCH

Clinical Utility of Central and Peripheral Airway Nitric Oxide in Children with Different Types of Allergic Asthma

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Background: Fractional exhaled nitric oxide has been widely used as a biomarker of airway inflammation. By measuring nitric oxide concentrations at different exhalation flow rates, it is possible to assess inflammation in various segments of the respiratory tract. This study hypothesized that FeNO could serve as a valuable tool for evaluating airway inflammation in children with different types of allergic asthma.

Methods: This retrospective single-center study included 487 children with asthma, categorized as inhalant-sensitized (n=238), food-sensitized (n=36), mixed-sensitized (n=181), and non-sensitized controls (n=32). Fractional exhaled nitric oxide was measured following ERS/ATS protocols, including $FeNO_{50}$ (flow rate 50 mL/s), $FeNO_{200}$ (200 mL/s), and CaNO (alveolar or peripheral airway NO concentration). Multivariable median regression was used to assess group differences after adjusting for age, sex, BMI, inhaled corticosteroid (ICS) use, rhinitis, and recent respiratory infection.

Results: $FeNO_{50}$ and $FeNO_{200}$ levels were significantly higher in both the inhalation allergen and mixed allergen groups compared to the control and food allergen groups (all adjusted $P < 0.01$). For instance, $FeNO_{50}$ showed an adjusted median difference of −5.00 ppb (95% CI: −8.50, −2.00; P =0.003) between the control group and inhalations. CaNO levels did not differ significantly across groups ($P = 0.133$).

Conclusion: $FeNO_{50}$ and $FeNO_{200}$ levels were significantly higher in inhalant- and mixed-sensitized children compared with food-sensitized or non-sensitized controls, indicating stronger type-2 inflammatory features. CaNO showed no significant difference across groups after adjustment. These findings highlight FeNO as a potential biomarker associated with airway inflammation in sensitized asthma; however, further prospective studies are warranted for confirmation.

Keywords: allergic asthma, allergen, fractional exhaled nitric oxide, airway inflammation

Introduction

Allergens play a critical role in triggering and exacerbating pediatric asthma, and allergic asthma is the main type or phenotype of asthma in children, especially in school-age children, accounting for 60% ~ 80% of asthma in China.[1] The fundamental pathology of asthma is characterized by chronic airway inflammation, primarily driven by type 2 inflammation.[2] When allergens stimulate Th2 cells, IgE production leads to the release of inflammatory mediators, activating the IgE immune pathway and resulting in chronic airway inflammation. Fractional exhaled nitric oxide (FeNO) is non-invasive, safe and easily measurable biomarker that reflects type 2 airway inflammation. $FeNO_{50}$ refers to FeNO measured at a 50 mL/s flow rate mainly reflects large airway inflammation. $FeNO_{200}$ was measured at 200mL/s which indicates inflammation in peripheral airways. Peripheral airway or alveolar NO (CaNO) is calculated at multiple flow rate through two-compartment model, and CaNO reflects inflammation in peripheral airway or alveolar.

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These FeNO values can predict the efficacy of glucocorticoids and type 2 inflammation-targeted therapies in asthmatic children.[3,4] Furthermore, identifying the type of allergens is crucial for diagnosis and treatment as well as monitoring the prognosis in pediatric allergic asthma.[5] However, few research explored the relationship between different allergy types and eNO levels in pediatric asthma. This retrospective study aimed to investigate the utility of FeNO in assessing airway inflammation by analyzing $FeNO_{50}$, $FeNO_{200}$, and CaNO levels in asthmatic children over 6 years old with different allergy types.

Methods

Objective

It was a retrospective study involving asthmatic children who visited the Respiratory Department of Children's Hospital, School of Medicine, Shanghai Jiaotong University from March 2020 to March 2023. Our study complies with the Declaration of Helsinki.

Inclusion Criteria. a. All patients satisfied the diagnostic criteria outlined in the Global Initiative for Asthma,[6] who aged over 6 years old with detailed allergen information. b. All patients were able to cooperate with the tests of FeNO50, FeNO200 and CaNO.

Exclusion criteria (fulfilling any one of the following). a. clinical or laboratory examination results indicating the presence of severe systemic diseases or any diseases that could potentially interfere with the objectives of this study; b. patients with intrapulmonary or extrapulmonary diseases such as pneumonia, cystic fibrosis or bronchiectasis of the lung; c. those for whom complete clinical data information could not be obtained.

Grouping and Data Collection

Participants were divided into four groups: inhalant allergen group, food allergen group, mixed group (combination of inhalant and food allergies) and allergen-negative control group. The control group consisted of allergen-negative asthma patients, defined as individuals with negative results for serum allergen-specific IgE (<0.35 kU/L) and without a clinical history of allergen-related symptoms. No healthy individuals were included in this study.

Medical records were collected and demographic and clinical data were recorded, including age, gender, weight, height, BMI; history of any episodes within the last 4 weeks, presence of infections, rhinitis history, and results of measurement of eNO indicators ($FeNO_{50}$, $FeNO_{200}$, CaNO).

Asthma control was evaluated using a comprehensive approach combining: symptom frequency (daytime and nighttime), pulmonary function follow-up results (FEV_1, PEF), physician assessment documented in medical records, and medication adjustment history (controller and rescue medication use). This integrated assessment allowed accurate stratification of patients, capturing both overt exacerbations and subclinical or partially controlled asthma.

Allergen Detection

The allergen detection was conducted by personnel from the Allergy Testing Laboratory at Children's National Medical Center, Shanghai Jiao Tong University School of Medicine. Serum allergen-specific IgE levels were measured using the ImmunoCAP system (Phadia, Sweden), with values ≥0.35 kU/L considered positive. There were 13 types of inhalant allergens were included: Dermatophagoides, house dust, cat hair/dander, dog hair/dander, cockroach, short ragweed, mugwort, amaranth, humulus scandens, mixed grass (eg, ryegrass), mulberry, mold mix (eg, Aspergillus fumigatus), and tree pollen (eg, elm, sycamore). Food allergens encompassed 15 types, including pineapple, chicken egg white, cow's milk, beef, mutton, shrimp, crab, codfish, salmon, shellfish, lobster/scallop, cashew nut, peanut, soybean, and mango, as shown in Figure 1A.

Skin prick testing (SPT) was not performed because all participants were children, and SPT results are more likely to be influenced by external factors such as environmental conditions, recent antihistamine use, and skin reactivity. In contrast, serum-specific IgE testing provides quantitative, objective, and reproducible data, allowing for more accurate assessment of sensitization level and longitudinal comparison. Allergic asthma was defined as asthma with at least one positive allergen-specific IgE result in conjunction with a relevant clinical history.

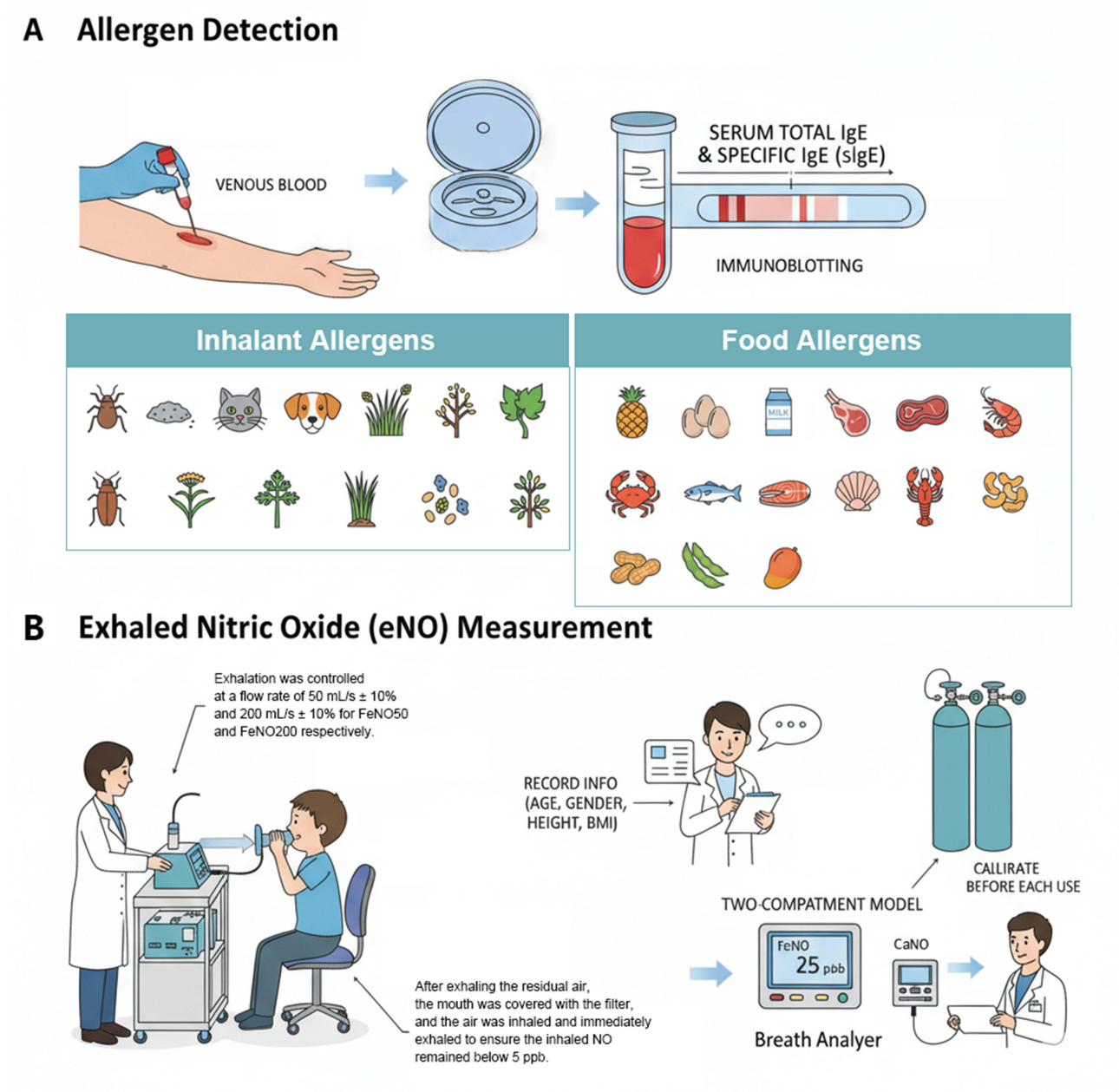

Figure 1 Allergen Detection and eNO measurement.

eNO Measurement

Exhaled nitric oxide (eNO) was measured by a Nano coulomb Breath Analyzer (Sunvou-CA2122, Wuxi, China) according to the recommendations of ERS/ATS. Patients were instructed to fast for 2 hours prior to the test, refrain from vigorous exercise and pulmonary function assessment. Prior to the test, the operator explained the procedure and precautions to the patients, and recorded their information including age, gender, height, BMI. During the test, patients were seated and held the inhalation filter. After exhaling the residual air, the mouth was covered with the filter, and the air was inhaled and immediately exhaled to ensure the inhaled NO remained below 5 ppb. Exhalation was controlled at a flow rate of 50 mL/s ± 10% and 200 mL/s ± 10% for $FeNO_{50}$ and $FeNO_{200}$ respectively. Once the exhalation met the requirements, the machine automatically initiated the test analysis and displayed the results. CaNO is calculated at multiple flow rate through two-compartment model, based on paired FeNO values obtained at 50 mL/s and 200 mL/s. To

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ensure instrument accuracy, dedicated personnel calibrated the instrument before each use with two standard gas mixtures of NO concentrations at 60 ppb and 250 ppb, as shown in Figure 1B.

Statistical Methods

This study utilized R Studio (version 2023.09.0) running R language version 4.3.1. The tidyverse package was employed for data cleaning, merging, and filtering. The ggplot2 package was utilized for graphical representation. For quantitative data, Shapiro–Wilk test was used to assess normality. Continuous variables were presented as median with interquartile range M[Q1-Q3]. Non-continuous variables were described as sample size/total sample size (percentage). For comparisons of quantitative data between groups, the non-parametric Kruskal–Wallis test was applied. When the Kruskal–Wallis test showed a statistically significant difference ($P < 0.05$), post-hoc pairwise comparisons were performed using the Mann–Whitney *U*-test with Bonferroni correction to adjust for multiple comparisons. These analyses were facilitated by the dunn. Test and rcompanion R packages. For qualitative data, statistical methods included Fisher's exact test and Pearson's chi-square test, chosen based on variable characteristics. *P* value <0.05 was considered statistically significant. Statistical analyses were primarily conducted using base R and packages such as dunn. Test and rcompanion for specific non-parametric post-hoc tests.

Results

Demographic Characteristics

487 pediatric children were included in the study, comprising the Inhalant Group with 238 cases (48.9%), the Food Group with 36 cases (7.4%), the Mixed Group with 181 cases (37.1%), and the Control Group with 32 cases (6.6%). Among them, there were 328 males (67%) and 159 females (33%).

The overall positive rate of allergen in asthmatic children in this sample was 93.4%, with a single inhalant allergen being more prevalent, accounting for 48.9%, significantly higher than the positive rate of a single food allergen (7.4%).

The distribution of gender exhibited a statistically significant difference among asthma groups classified by different allergy types ($P <0.001$), as illustrated in Table 1.

Conversely, no statistically significant differences were observed in age, weight, height, or BMI among the various groups ($P >0.05$).

Furthermore, through the statistics of the distribution of allergens, we found that the frequency of inhaled allergens was generally higher than that of food allergens. Dust mites (270) had the highest frequency of inhaled allergens, followed by cat dander (68), and mulberry, mugwort and amaranth had the lowest frequency, all being 3. The most frequent food allergens were milk (115), followed by egg white (44), while pineapple and lamb had the lowest frequency (1), as shown in Figure 2.

Fractional Exhaled Nitric Oxide Among Different Allergy Types

Overall, $FeNO_{50}$ and $FeNO_{200}$ showed significant differences ($P <0.001$) among the groups, as illustrated in Table 2. The median $FeNO_{50}$ in the control group was 15.00 [11.00–17.00]. Relative to the control group, $FeNO_{50}$ levels were 20.00

Table 1 Comparison of Demographic Characteristics Among Different Allergen Groups

Parameters	Control Group (n=32)	Inhalation Allergen Group (n=238)	Food Allergen Group (n=36)	Mixed Allergen Group (n=181)	P value
Age	9.00 [8.00–11.25]	10.00 [8.00–12.00]	9.50 [8.00–12.00]	10.00 [8.00–11.00]	0.703
Sex	18/32 (56.2%)	140/238 (58.8%)	27/36 (75.0%)	143/181 (79.0%)	0.000***
Male	18/32 (56.2%)	140/238 (58.8%)	27/36 (75.0%)	143/181 (79.0%)	0.000***
Female	14/32 (43.8%)	98/238 (41.2%)	9/36 (25.0%)	38/181 (21.0%)	0.000***
Height (cm)	133.65 [125.83–147.95]	136.30 [124.40–150.27]	138.85 [129.07–149.80]	135.00 [126.00–147.70]	0.575
Weight (kg)	32.00 [25.38–35.50]	31.50 [26.77–43.00]	36.85 [27.23–46.48]	33.00 [25.80–44.00]	0.426
BMI (kg/m^2)	16.35 [15.67–20.30]	17.60 [15.80–20.70]	18.35 [17.50–19.40]	18.00 [16.00–20.60]	0.351

Notes: Continuous variables are presented as median [Q1-Q3].Categorical variables are presented as n/N (%).Statistical tests used: Kruskal–Wallis test for continuous variables, Chi-square test for categorical variables. *** means P < 0.001.

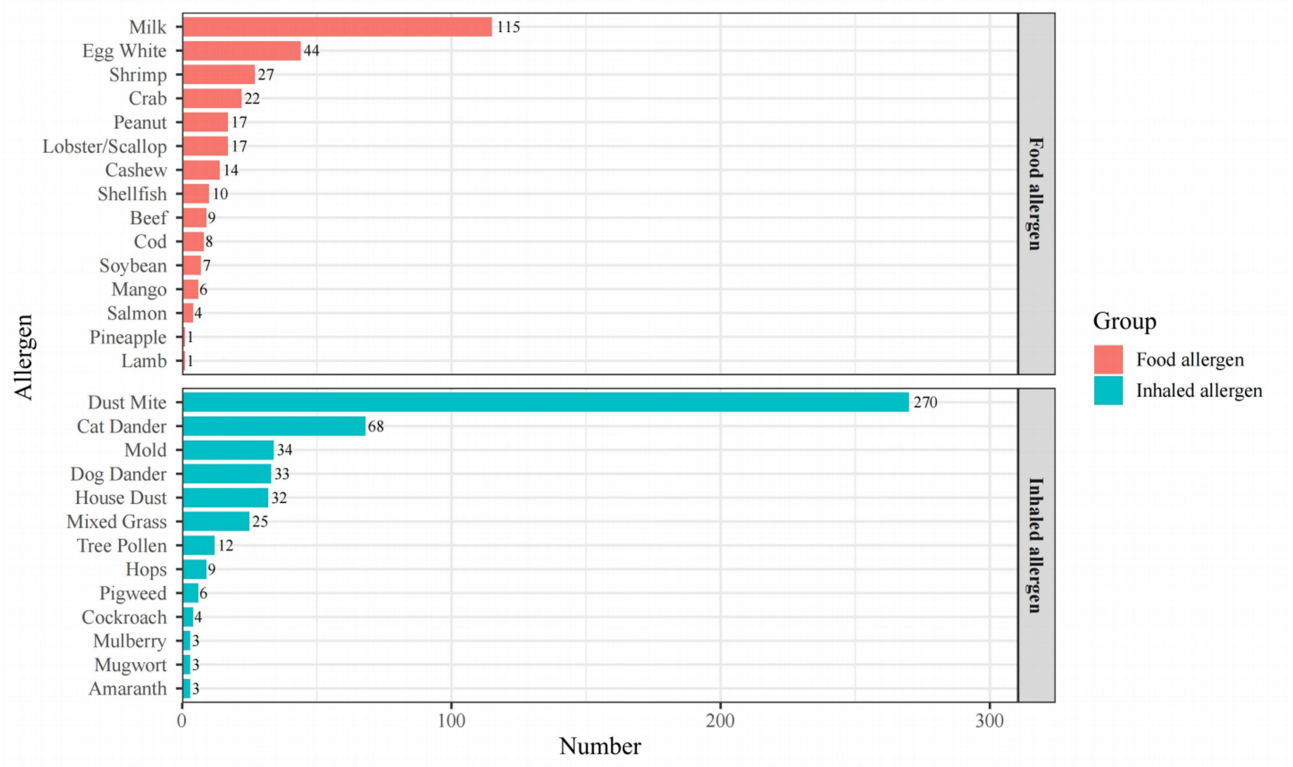

Figure 2 Distribution of different types of allergens.

ppb [13.00–31.00] in the Inhalation Allergen Group (adjusted median difference = −5.00 ppb, 95% CI: −8.50, −2.00; *P* =0.003), and 21.00 ppb [14.00–33.00] in the Mixed Allergen Group (adjusted median difference = −6.00 ppb, 95% CI: −10.00, −2.00; *P*=0.002). Furthermore, $FeNO_{50}$ in the Food Allergen Group, which recorded 12.00 ppb [11.00–19.25], showed significant differences when compared to the Inhalation Allergen Group (adjusted median difference = 8.00 ppb, 95% CI: 2.00, 10.00; *P*=0.003) and the Mixed Allergen Group (adjusted median difference = 9.00 ppb, 95% CI: −12.00, −2.50; *P* =0.002). A similar pattern was observed for $FeNO_{200}$. The median $FeNO_{200}$ in the control group was 9.00 [7.00–11.00]. When compared to the control group, $FeNO_{200}$ registered 12.00 ppb [8.00–17.00] in the Inhalation Allergen Group (adjusted median difference = −3.00 ppb, 95% CI: −4.00, −0.50; *P* =0.004) and 12.00 ppb [9.00–17.00] in the Mixed Allergen Group (adjusted median difference = −3.00 ppb, 95% CI: −5.00, −1.50; *P* =0.001). The Food Allergen Group presented a FeNO200 of 9.00 ppb[7.00–11.25], which was notably distinct from the Inhalation Allergen Group (adjusted median difference = 3.00 ppb, 95% CI: 1.00, 4.50; *P* =0.003) and the Mixed Allergen Group (adjusted median difference = −3.00 ppb, 95% CI: −5.00, −2.00; *P* =0.001). This suggests that airway inflammatory responses are more pronounced in children with inhalant and mixed allergies compared to those with single allergen positivity, as

Table 2 Comparison of Exhaled Nitric Oxide Parameters Among Different Allergen Groups

Exhaled Nitric Oxide Parameters	Control Group (n=32)	Inhalation Allergen Group (n=238)	Food Allergen Group (n=36)	Mixed Allergen Group (n=181)	P value
FeNO50(ppb)	15.00 [11.00–17.00]	20.00 [13.00–31.00]	12.00 [11.00–19.25]	21.00 [14.00–33.00]	0.000***
FeNO200(ppb)	9.00 [7.00–11.00]	12.00 [8.00–17.00]	9.00 [7.00–11.25]	12.00 [9.00–17.00]	0.000***
CaNO(ppb)	6.85 [5.00–8.27]	7.60 [5.10–10.47]	6.20 [4.75–9.05]	7.50 [5.30–9.60]	0.133

Notes: Data are presented as median [Q1-Q3] for all continuous variables. Statistical tests used: Kruskal–Wallis test for group comparisons of continuous variables. *** means P < 0.001.

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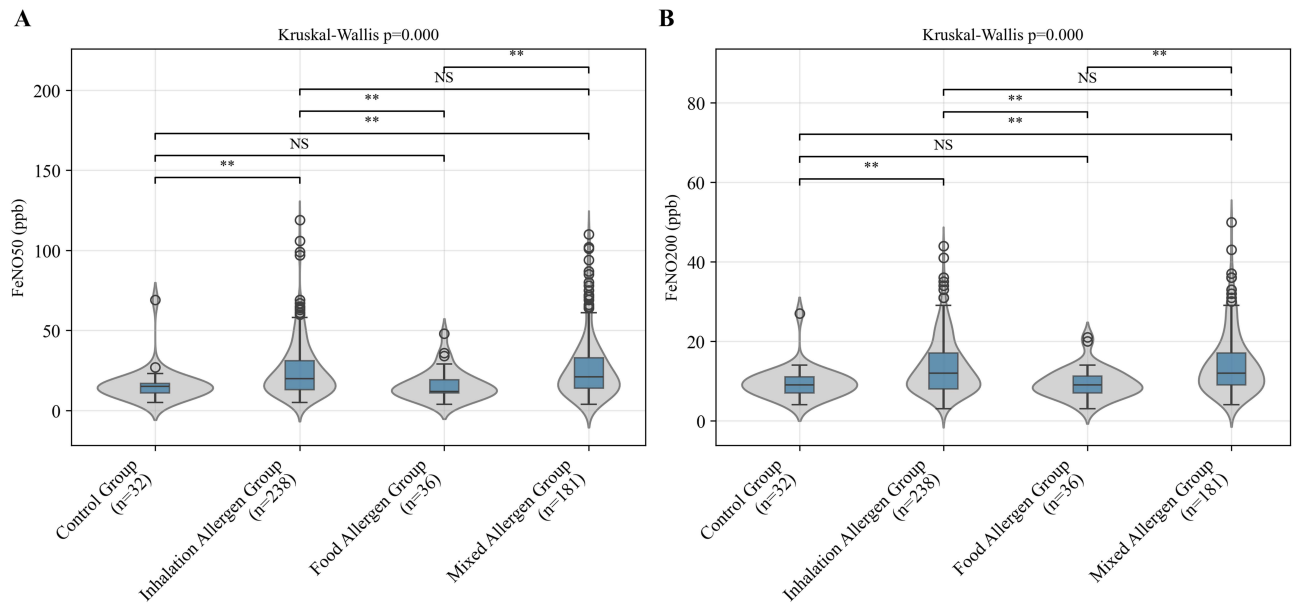

Figure 3 Differences between $FeNO_{50}$ (**A**), $FeNO_{200}$ (**B**) in different types of asthma subgroups.
Notes: Post-hoc pairwise comparisons were performed using Mann–Whitney *U*-test with Bonferroni correction.P-values were adjusted for multiple comparisons using Bonferroni correction. ** means P < 0.01, *** means P < 0.001, NS means not statistically significant.

illustrated in Figure 3A and B. However, no statistically significant difference was found in CaNO levels between the various allergy groups and the control group (P >0.05).

Stratified Analyses

Considering the potential differences in disease severity and airway inflammation levels among various types of asthmatic children under different conditions, further stratified analyses were conducted to examine potential influencing factors such as recent asthma control status, infections and history of rhinitis, including: (1) Stratification by asthma control status: no episode, well-controlled (N=327), and with episodes, poorly controlled (N=160); (2) Stratification by recent infection: no infection (N=425) and with infection (N=62); (3) Stratification by history of rhinitis: no history of rhinitis (N=121) and with history of rhinitis (N=366), as illustrated in Table 3.

Among poorly controlled asthmatic children, $FeNO_{50}$ demonstrated statistically significant overall differences among groups (P =0.005). Specifically, $FeNO_{50}$ levels were 21.00 ppb [15.00–34.50] in the Inhalation Allergen Group and 22.00 ppb [16.00–34.75] in the Mixed Allergen Group, both significantly higher than those in the Food Allergen Group (12.00 ppb[11.00–17.50]) (Inhalation Allergen vs Food Allergen: adjusted median difference = 9, 95% CI: 2.00, 13.00; P =0.026; Food Allergen vs Mixed Allergen: adjusted median difference = −10, 95% CI: −17.00, −3.00; P =0.019). No significant differences were found for FeNO200 (P =0.064) or CaNO (P =0.640) in this group, as shown in Figure 4A.

Among well-controlled asthmatic children, $FeNO_{50}$ levels in the Inhalation Allergen Group (19.00ppb [13.00–30.50]) and Mixed Allergen Group (20.00 ppb [13.00–32.00]) were significantly higher than those in the Control Group (14.00ppb [9.75–17.00]) (Control vs Inhalation Allergen: adjusted median difference = −5 ppb, 95% CI: −9.50, −1.00; P =0.013; Control vs Mixed Allergen: adjusted median difference = −6 ppb, 95% CI: −11.00, −2.00; P =0.013). Similarly, $FeNO_{200}$ levels in the Inhalation Allergen Group (11.00 ppb [8.00–16.00]) and Mixed Allergen Group (12.00 ppb [9.00–16.00] were significantly higher than the Control Group (9.00ppb [7.00–10.25]) (Control vs Inhalation Allergen: adjusted median difference = −2 ppb, 95% CI: −4.00, −1.00; P =0.019; Control vs Mixed Allergen: adjusted median difference = −3 ppb, 95% CI: −5.00, −1.00; P =0.006). Moreover, $FeNO_{200}$ levels in the Food Allergen Group (8.00 ppb [7.00–10.00] were significantly lower than the Inhalation Allergen Group (adjusted median difference = −3 ppb, 95% CI: −5.00, −1.00; P =0.005) and the Mixed Allergen Group (adjusted median

Table 3 Comparison of Exhaled Nitric Oxide Parameters Under Different Clinical Conditions

Condition	Parameter	Control Group (n=32)	Inhalation Allergen Group (n=238)	Food Allergen Group (n=36)	Mixed Allergen Group (n=181)	P value
Asthma in Control (N=327)	FeNO50(ppb)	14.00 [9.75–17.00]	19.00 [13.00–30.50]	15.00 [12.00–20.00]	20.00 [13.00–32.00]	0.002**
	FeNO200(ppb)	9.00 [7.00–10.25]	11.00 [8.00–16.00]	8.00 [7.00–10.00]	12.00 [9.00–16.00]	0.000***
	CaNO(ppb)	6.85 [4.50–8.27]	7.50 [5.05–10.70]	5.70 [4.00–6.20]	7.80 [5.30–9.55]	0.014*
Asthma out of Control (N=160)	FeNO50(ppb)	17.00 [12.00–18.25]	21.00 [15.00–34.50]	12.00 [11.00–17.50]	22.00 [16.00–34.75]	0.005**
	FeNO200(ppb)	10.50 [7.75–12.00]	12.00 [9.00–17.00]	10.00 [8.50–12.50]	13.00 [8.25–17.75]	0.064
	CaNO(ppb)	7.20 [6.05–8.28]	7.80 [5.25–10.25]	7.60 [6.80–10.90]	7.00 [5.53–10.22]	0.640
With no infection recently (N=425)	FeNO50(ppb)	15.00 [11.25–17.00]	20.00 [13.00–31.75]	12.00 [11.00–19.25]	22.00 [14.00–34.00]	0.000***
	FeNO200(ppb)	9.00 [7.25–11.00]	11.00 [8.00–17.00]	9.00 [7.00–11.00]	13.00 [9.00–17.00]	0.000***
	CaNO(ppb)	7.10 [4.80–8.43]	7.40 [5.00–10.10]	6.15 [4.52–7.62]	7.50 [5.10–9.60]	0.140
With infection recently (N=62)	FeNO50(ppb)	14.50 [11.25–17.75]	18.50 [12.00–29.00]	14.00 [10.00–21.25]	14.00 [10.00–22.25]	0.371
	FeNO200(ppb)	9.50 [7.25–11.00]	13.00 [9.75–17.00]	11.50 [9.25–14.75]	9.50 [7.75–16.00]	0.168
	CaNO(ppb)	6.30 [5.95–7.55]	10.00 [7.42–12.00]	10.35 [8.88–11.65]	8.75 [5.78–10.62]	0.181
Without history with rhinitis (N=121)	FeNO50(ppb)	17.00 [13.00–18.00]	19.00 [14.00–27.00]	13.50 [11.00–19.50]	18.00 [12.00–30.00]	0.359
	FeNO200(ppb)	11.00 [8.00–12.00]	10.00 [8.00–14.00]	8.00 [7.00–9.25]	9.00 [7.00–14.50]	0.233
	CaNO(ppb)	8.10 [6.20–8.80]	5.00 [3.30–7.80]	5.95 [3.85–6.70]	5.60 [3.50–8.50]	0.354
With history with rhinitis (N=366)	FeNO50(ppb)	14.00 [10.00–17.00]	21.00 [13.00–32.00]	12.00 [10.50–18.50]	22.00 [14.00–34.75]	0.000***
	FeNO200(ppb)	9.00 [7.00–10.50]	12.00 [8.00–17.00]	9.50 [7.00–12.25]	13.00 [10.00–17.00]	0.000***
	CaNO(ppb)	6.50 [4.30–7.90]	8.40 [6.00–10.90]	6.60 [5.40–9.85]	8.15 [6.10–11.00]	0.017*

Notes: Data are presented as median [Q1-Q3] for all continuous variables. Each condition shows the comparison of three parameters across four allergen groups. Statistical tests used: Kruskal–Wallis test for group comparisons of continuous variables. * means P < 0.05, ** means P < 0.01, *** means P < 0.001.

difference = −4 ppb, 95% CI: −6.00, −2.00; P =0.002). Differences in CaNO levels were observed among the groups with confirmed positive allergens, with both the Inhalation Allergen Group (7.50 ppb [5.05–10.70]) and Mixed Allergen Group (7.80 ppb [5.30–9.55]]) showing significantly higher CaNO levels than the Food Allergen Group (5.70 ppb [4.00–6.20]) (Inhalation Allergen vs Food Allergen: adjusted median difference = 1.8, 95% CI: 0.90, 3.60; P =0.029; Food Allergen vs Mixed Allergen: adjusted median difference = −2.1, 95% CI: −4.00, −1.00; P =0.023), as shown in Figure 4B–D.

Among asthmatic children without recent infection, $FeNO_{50}$ levels were significantly higher in the Inhalation Allergen Group (20.00 ppb[13.00–31.75]) and Mixed Allergen Group (22.00 ppb[14.00–34.00]) compared to the Control Group (15.00 ppb[11.25–17.00]) (Control vs Inhalation Allergen: adjusted median difference = −5, 95% CI: −9.00, −2.00; P =0.012; Control vs Mixed Allergen: adjusted median difference = −7, 95% CI: −11.00, −3.00; P =0.003). Both the Inhalation Allergen Group and Mixed Allergen Group also showed significantly higher $FeNO_{50}$ levels than the Food Allergen Group (12.00 ppb [11.00–19.25]) (Inhalation Allergen vs Food Allergen: adjusted median difference = 8, 95% CI: 2.00, 10.00; P =0.005; Food Allergen vs Mixed Allergen: adjusted median difference = −10, 95% CI: −12.00, −3.50; P =0.001).

Similarly, $FeNO_{200}$ levels were significantly higher in the Inhalation Allergen Group (11.00 ppb [8.00–17.00]] and Mixed Allergen Group (13.00 ppb [9.00–17.00]) compared to the Control Group (9.00 ppb [7.25–11.00]) (Control vs Inhalation Allergen: adjusted median difference = −2, 95% CI: −4.00, −0.00; P =0.029; Control vs Mixed Allergen: adjusted median difference = 4, 95% CI: −5.00, −2.00; P =0.002). Furthermore, $FeNO_{200}$ levels in the Inhalation Allergen Group were significantly higher than in the Food Allergen Group (9.00 ppb [7.00–11.00]] (adjusted median difference = 2, 95% CI: 1.00, 4.50; P =0.004), and the Mixed Allergen Group had significantly higher $FeNO_{200}$ levels than the Food Allergen Group (adjusted median difference = 4, 95% CI: 2.00,6.00; P <0.001). No significant differences were observed for CaNO (P =0.140), as shown in Figure 4E and F.

In asthmatic children with recent infection or without a history of rhinitis, no statistically significant differences were found in eNO levels among groups (P >0.05).

Among asthmatic children with a history of rhinitis, $FeNO_{50}$ levels were significantly higher in the Inhalation Allergen Group (21.00 ppb [13.00–32.00]) and Mixed Allergen Group (22.00[14.00–34.75]) compared to the Control Group (19.00 ppb [13.00–32.00]) (Control Group vs Inhalation Allergen Group: adjusted median difference = −7, 95% CI: −11.00, −3.00; P =0.002; Control Group vs Mixed Allergen Group: adjusted median difference = −8, 95% CI:

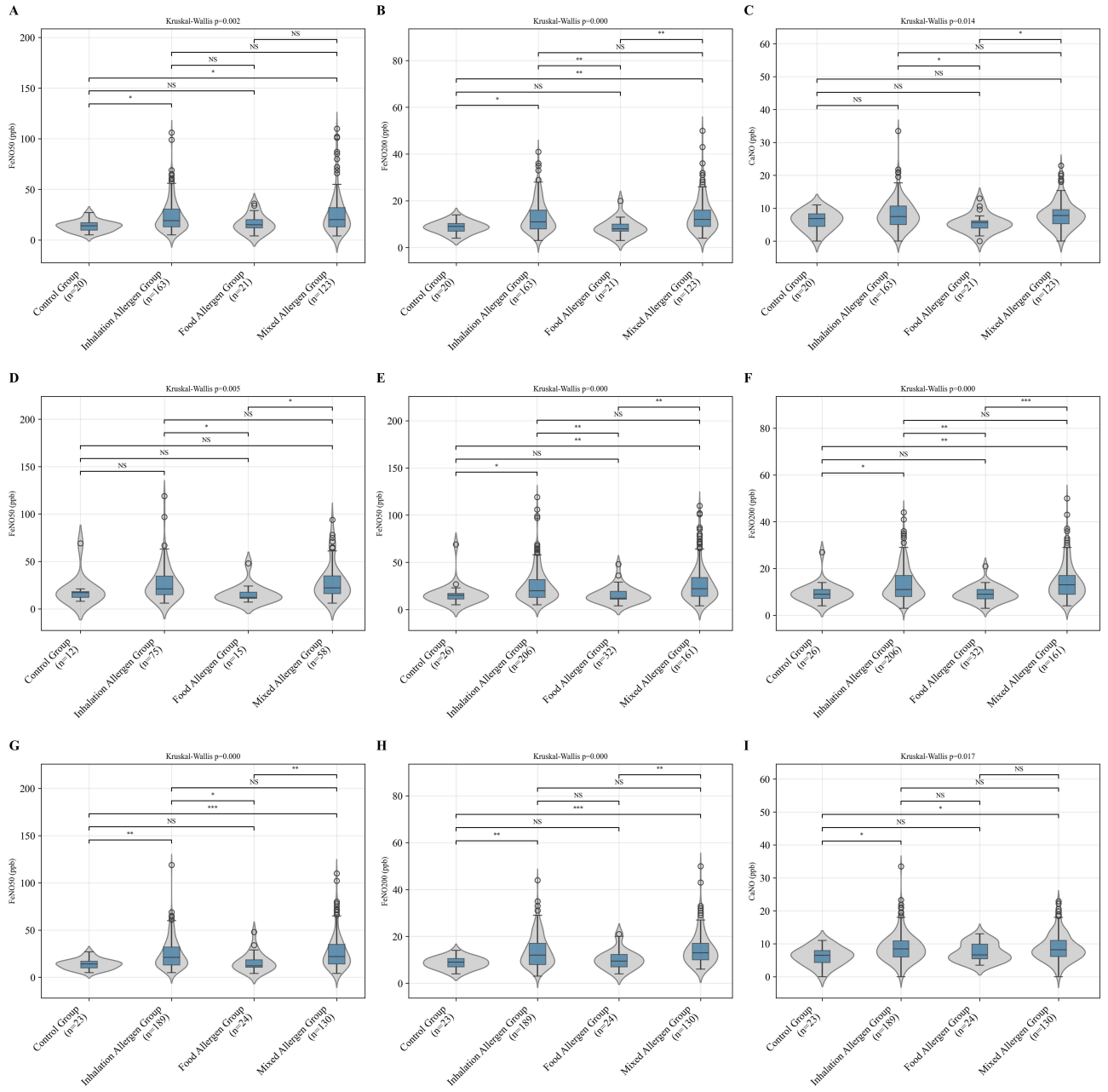

Figure 4 Analysis of the differences in eNO of children with different allergic types of asthma under different triggering factors. Differences in $FeNO_{50}$ (**A**) among children with different types of asthma with poor control. Differences in $FeNO_{50}$ (**B**), $FeNO_{200}$ (**C**) and CaNO (**D**) among children with different types of asthma with good control. Analysis of differences in $FeNO_{50}$ (**E**) and $FeNO_{200}$ (**F**) and among children with different types of asthma without infection. Analysis of differences in $FeNO_{50}$ (**G**), $FeNO_{200}$ (**H**) and CaNO (**I**) among children with different types of asthma with a history of rhinitis.

Notes: Post-hoc pairwise comparisons were performed using Mann–Whitney *U*-test with Bonferroni correction. P-values were adjusted for multiple comparisons using Bonferroni correction. * means P < 0.05, ** means P < 0.01, *** means P < 0.001, NS means not statistically significant.

−13.00, −3.50; P <0.001). Both the Inhalation Allergen Group and Mixed Allergen Group also showed significantly higher $FeNO_{50}$ levels than the Food Allergen Group (12.00 ppb [10.50–18.50]) (Inhalation Allergen Group vs Food Allergen Group: adjusted median difference = 9, 95% CI: 1.00, 10.50; P =0.017; Food Allergen Group vs Mixed Allergen Group: adjusted median difference = −10, 95% CI: −13.00, −3.50; P =0.004).

Similarly, $FeNO_{200}$ levels were significantly higher in the Inhalation Allergen Group (12.00 ppb[8.00–17.00]) and Mixed Allergen Group (13.00 ppb[10.00–17.00]) compared to the Control Group (9.00 ppb [7.00–10.50]) (Control Group vs Inhalation Allergen Group: adjusted median difference = 3, 95% CI: −5.00, −2.00; P =0.001; Control Group vs

Mixed Allergen Group: adjusted median difference = 4, 95% CI: −6.01, −2.00; P <0.001). $FeNO_{200}$ levels in the Food Allergen Group (9.50 ppb [7.00–12.25]) were significantly lower than the Mixed Allergen Group (adjusted median difference = −3.5, 95% CI: −5.50, −1.00; P =0.004).

Regarding CaNO, levels were significantly higher in the Inhalation Allergen Group (8.40 ppb [6.00,10.90] and Mixed Allergen Group (8.15 ppb [6.10–11.00] only when compared to the Control Group (6.50 ppb [4.30–7.90]) (Control Group vs Inhalation Allergen Group: adjusted median difference = −1.9, 95% CI: −3.40, −0.40; P =0.035; Control Group vs Mixed Allergen Group: adjusted median difference = −1.65, 95% CI: −3.30, −0.35; P =0.035). There was no significant difference in CaNO levels between the Food Allergen Group (6.60 ppb [5.40–9.85]) and the Control Group, despite an overall group difference (P =0.017), as shown in Figure 4G–I.

Discussion

A potential limitation of this study is the absence of skin prick testing (SPT). However, in pediatric populations, serum allergen-specific IgE testing is considered a reliable alternative with higher reproducibility and less interference from environmental or pharmacologic factors. Moreover, its quantitative nature enables precise evaluation of sensitization degree and longitudinal follow-up, thereby reducing potential misclassification of allergic status. There are significant differences in eNO levels among asthmatic children with different types of allergies. In this study, the airway inflammation indicators (such as $FeNO_{50}$ and $FeNO_{200}$) in the inhaled allergen group were significantly higher than those in the food allergen group and the control group, which may be closely related to the pathogenesis of childhood asthma. The inhalant allergen enters the body through the respiratory tract, the airway mucosa produces IgE antibody after contact with the corresponding allergen, and is in the sensitization state, when the allergen re-enters the airway of asthmatic children, it binds specifically with IgE antibody and produces a lot of inflammatory mediators such as histamine and leukotriene. Then the type 2 cytokine IL-13 upregulates inducible NO synthase, leading to an increase in NO production by airway epithelial and inflammatory cells. Airway inflammation can be measured by sputum induction and bronchial biopsy, but more and more studies have shown that NO plays a key regulatory role in type 2 airway inflammation such as asthma and allergic rhinitis. Therefore, although FeNO cannot replace sputum eosinophils in assessing airway inflammation, it can help identify type 2 airway inflammation. IgE-mediated food allergy is also very common, food allergens enter children's body from the digestive tract because the children's gastrointestinal tract is not fully developed, the gastrointestinal barrier function is not perfect, and the mucosal permeability is high, food allergen components are easy to enter the body fluids. The abnormal production of IgE and the activation of IgE immune pathway after exposure to food allergens in the sensitization stage are the key mechanisms.[7] On the one hand, food allergens can induce airway hyperresponsiveness and lead to asthma attack. On the other hand, systemic allergic reactions induced by food allergy are also more likely to be complicated by respiratory symptoms.[8] Different from inhalation allergy, food allergy will rapidly trigger symptoms in multiple parts such as gastrointestinal tract, skin and respiratory tract, and systemic reactions of varying severity may also occur.[8] However, eNO mainly detects airway inflammation levels, which may explain the stronger inflammatory response of eNO in asthmatic children in the inhalation group than in the food group in this study. Although the food allergen panel used in this study covered the most common allergens among Chinese children, some allergens such as egg yolk and wheat were not tested, which may have led to underestimation of food sensitization prevalence.

The eNO contains two parts including large airway NO and small airway NO, $FeNO_{50}$ contains 75% and 25% respectively, while $FeNO_{200}$ contains 25% and 75% respectively. CaNO is the peripheral small airway/alveolar NO concentration calculated at multiple flow rate through two-compartment model. The three types of eNO indicators reflect the level of airway inflammation in different parts. The specificity and sensitivity of eNO in diagnosing asthma vary greatly among different research reports, partly due to differences in the critical and reference values evaluated. ATS guidelines define high, medium and low FeNO levels in children as >35 ppb, 20–35 ppb and <20ppb.[9] Considering some differences in asthma incidence and reference values between foreign countries and China, the national multi-center study in 2010–2012 concluded that the 95% confidence interval of FeNO value for children aged 6–14 years was 5–24 ppb, and the 95% confidence interval of FeNO value for people over 15 years old was 5–30 ppb.[10] A recent multi-center study in China concluded that the normal reference range of FeNO for Chinese school-age children and adolescents aged

6–18 years was 1.0–38.2 ppb, and it was suggested that 16 ppb should be used as the clinical cut-off point for the identification of airway inflammation in the 12–18 years old group. For children aged 6–11 years, FeNO cut-off value would decrease by 1 ppb for every 1 year of age decrease.[11] The normal ranges of FeNO50, FeNO200 and CaNO indexes in healthy children aged 6–18 years in Jinan were 3.0–35.0 ppb, 2.0–13.3 ppb, 0.5–8.5 ppb and 29.3–863.4 ppb, respectively. $FeNO_{50}$ is related to age, height and BMI. $FeNO_{200}$ is related to gender and height, while CaNO is not related to age, height and BMI.[12] This study found that FeNO200 and CaNO had higher clinical value in evaluating stable control and no infection in asthmatic children with different allergic types, while $FeNO_{50}$ was significantly higher in the inhalation group and the mixed group than that in the food group regardless of asthma control. This may explain that there is a negative correlation between the disease control and the level of airway inflammation in children with asthma, but there are differences between the sites of airway inflammation and the overall control level.

Children with both asthma and rhinitis have elevated airway inflammatory indicators due to increased nitric oxide production by the nasal mucosa. For children with upper and lower airway diseases such as rhinitis and asthma, the level of eNO in the inhaled allergen group was significantly higher than that in the food allergen group in this study, which was consistent with the research results of Ildiko et al.[13] FeNO can predict the occurrence of asthma in patients with persistent house dust mite allergic rhinitis. Adult patients with a longer duration of rhinitis symptoms and a higher FeNO level are at a greater risk of asthma.[14] Therefore, in clinical assessment of airway inflammation, attention should be paid to the joint evaluation of the upper and lower airways. A multi-center cross-sectional study in Europe suggested that FeNO elevation interacts with perennial sensitization, while also emphasizing the concept of combined airway disease, suggesting that there is a correlation between upper and lower respiratory tract symptoms and inflammation, and emphasizing the sensitization effect.[15] The study further found that the detection rate of FeNO combined with allergen detection in children with recurrent wheezing was significantly better than that of the two alone,[16] which has important clinical application value for the diagnosis of early asthma. While asthma control was assessed comprehensively, some patients may still have elevated FeNO despite apparent symptom stability, reflecting ongoing subclinical airway inflammation.

FeNO is highly expressed in allergen-positive children. As a non-invasive biomarker of type 2 airway inflammation, higher FeNO in asthma patients is associated with increased iNOS mRNA in bronchial epithelium.[17] A cohort study suggested that FeNO levels were elevated in children with asthma, depending on the presence of aeroallergen sensitization.[18] $FeNO_{50}$ mainly reflects middle airway inflammation, $FeNO_{200}$ and CaNO mainly reflect small airway inflammation. CaNO combined with pulmonary function can also assist in the diagnosis of asthma with small airway dysfunction and monitor disease management.[19] Combined evaluation of airway inflammation levels in asthma is more conducive to reflect airway function in children with inhalation allergy and mixed allergic asthma. A cross-sectional study suggests that the relationship between childhood asthma and eNO is largely determined by allergen-specific IgE levels rather than genetic determination of blood eosinophils.[20] This may partially explain the clinical heterogeneity of the different allergy type groups, and the next step may be to incorporate allergen-specific IgE levels into multivariate omics studies. Defining the asthma phenotype can also help predict disease progression and biologic therapies to consider.[21]

Conclusions

This research highlights the significant role of different eNO in children with different types of allergic asthma. The concentration of $FeNO_{50}$ and $FeNO_{200}$ were significantly elevated in children with inhalant and mixed allergic asthma, which suggests these children may have more serious airway inflammation, and it may indicate that asthma has been poorly controlled recently. eNO can be used as a potential marker for disease surveillance. However, whether eNO is a biological indicator to distinguish allergic asthma from non-allergic asthma deserves further study. The clinical application value of CaNO in childhood asthma needs to be further studied. When $FeNO_{50}$ and $FeNO_{200}$ reference values are used, differences in allergic asthma in children caused by different allergen types should be considered. The pathogenesis pathways involved in the specific types of allergic asthma, such as inhalation allergy and food allergy, and the correlation with asthma endotype need further study.

Data Sharing Statement

The data that support the findings of this study are not publicly available due to their containing information that could compromise the privacy of research participants but are available from the corresponding author, Dong X. Y., via the Email address dongxy@shchildren.com.cn upon reasonable request.

Ethics Approval and Consent to Participate

Study approval statement: The study was approved by the Ethics Review Committee of the Children's Hospital of Shanghai/Shanghai Children's Hospital, Shanghai Jiao Tong University (Approval NO. 2021RY075-E01). The data are anonymous, and the requirement for informed consent was therefore waived.

Consent to participate statement: The study has been granted an exemption from requiring written informed consent by the Ethics Review Committee of the Children's Hospital of Shanghai/Shanghai Children's Hospital, Shanghai Jiao Tong University.

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This paper has been uploaded to Authorea as a preprint: https://www.authorea.com/users/847585/articles/1235357-clinical-utility-of-central-and-peripheral-airway-nitric-oxide-in-children-with-different-types-of-allergic-asthma

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work. All authors read and approved the final manuscript.

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Disclosure

The authors have no conflicts of interest to declare.

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