

Nitric oxide products and aldehydes in exhaled breath condensate in children with asthma

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Key messages

The role of aldehydes and oxides of nitrogen profiles in exhaled breath condensate in asthmatic children is still unclear.

We found that high levels of oxides of nitrogen and low levels of 4-hydroxynonenal in exhaled breath condensate are associated with allergic asthma in children.

Non-invasive detection of these markers may potentially be considered in the management of asthma.

To the Editor,

Diagnosis of asthma relies on symptoms and lung function test that may not reflect airway inflammation whose control is the cornerstone of asthma.¹ Many tools for assessing airway inflammation are invasive (bronchoalveolar lavage, endobronchial biopsy) or semi-invasive (sputum induction) and therefore unsuitable for routine use in children. In contrast, the measurement of inflammatory biomarkers in exhaled air or exhaled breath condensate (EBC) is non-invasive and easy to perform.² Fractional exhaled nitric oxide (FeNO) is the most studied exhaled biomarker. FeNO levels are related to airway eosinophils. When used with history, symptoms and lung function, FeNO can be helpful for asthma diagnosis and for modifying inhaled corticosteroid treatment.¹ However, FeNO levels are influenced by several allergic conditions including atopic eczema.³ EBC that mirrors lung lining fluid contains inflammatory and oxidative stress compounds.¹⁻⁹ Airway inflammation leads to an overproduction of reactive oxygen species (ROS), and the resulting oxidative damage is implicated in onset and persistence of asthma. ROS reacts with NO to form oxides of nitrogen (NOx), which can be a marker of oxidative stress in eosinophilic asthma. EBC NOx levels were elevated in asthmatic adolescents.⁴ Among NOx, inconsistent levels of nitrite

(NO₂) and nitrate (NO₃) have been found in asthmatic children.⁴⁻⁸ Oxidative stress processes involving peroxidation of membrane-bound polyunsaturated fatty acids form aldehydes, including malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE)⁵ that are considered markers of oxidative stress. In asthmatic children, conflicting findings on EBC MDA levels have been reported, and only one study compared EBC MDA levels with concentrations of other aldehydes, including 4-HNE.⁵ Studies comparing EBC levels of NO_x and aldehydes in asthmatic children are lacking. Our aim was to investigate whether aldehydes, MDA and 4-HNE or NO_x measured in EBC could be used to distinguish asthmatic children from healthy children.

This was a prospective study conducted in asthmatics aged 6–18 years, referred to the Department of Pediatrics, Center of Pulmonology and Pediatric Allergology of Bolzano Hospital, Italy between January and August 2020, and in healthy children who in the same period were referred to perform routine visits or investigations to the same department. Children who were consecutively enrolled in the study had asthma according to the Global Initiative for Asthma (GINA) criteria.¹ Exclusion criteria were severe asthma attack requiring hospitalization in the last 4 weeks, bronchial provocation test in the last week, intake of systemic corticosteroids, anti-leukotrienes, long-acting β 2-agonist or theophylline in the last 3 weeks, acute upper respiratory infection in the last 4 weeks, chronic respiratory infection, chronic cardiopulmonary disease, pneumonia in the last 4 weeks, nasal polyps, obesity, gastro-oesophageal reflux and aspirin-induced asthma. Healthy age-matched children without asthma and atopic diseases were included in the control group. Controls did not receive corticosteroids or had respiratory tract infections in the last 4 weeks. At recruitment, all children underwent physical examination and collection of an EBC sample for measuring NO_x, MDA and 4-HNE. All children with asthma underwent skin prick test (SPT), spirometry, reversibility test and FeNO measurement (https://osf.io/mr2cj/?view_only=18140c33a8554d79a23ba1b4983670b4). Study procedures were conducted approximately at the same hour in the morning and out of the relevant pollen allergen season. The pollen season was assessed using local data on pollen concentration. Atopy was defined as having at least one positive SPT result. Mono-sensitization was defined as sensitization to one inhalant allergen and poly-sensitization to 2 or more aeroallergens. The Italian version of Childhood Asthma Control Test (C-ACT) was administered to all asthmatics. The C-ACT score ranges from 0 to 27. A score ≤ 19 indicates uncontrolled asthma. The study was approved by the local ethics committee. All participants gave their written informed consent to participate in the study.

Thirty-three children with asthma were consecutively enrolled in the study (<https://osf.io/ewuh6/?pid=mr2cj>). There were 29 healthy age-matched controls, 38% females, 62% males. Within the asthmatic group, the C-ACT score, SPT results are depicted in Table 1. FeNO could be measured in all asthmatics (mean $\pm$ SEM (ppb) 14.23 $\pm$ 1.72). Nine (27%) asthmatics had FeNO values >20 ppb, 24 (73%) <20 ppb. One subject had FeNO >35 ppb. Levels of NO_x and aldehydes were determined in all EBC samples. NO_x levels were found to

be higher in asthmatics than in controls ($p = .001$). The ROC (receiver operating characteristics) analysis showed that the AUC (area under the curve) of NOx was 0.72, indicating that exhaled NOx had a good discriminating power to identify asthmatics from healthy subjects. The optimal cut-off point of NOx according to the Youden index was $0.64 \mu\text{M}$, which was associated with a sensitivity of 69% and a specificity of 66% (Figure 1, <https://osf.io/ex4n6/>). AUCs of MDA and 4-HNE were 0.41 and 0.30 respectively. In contrast, 4-HNE levels were higher in controls than in asthmatics ($p = .006$; Table 1). There was no difference between the two groups in MDA values (Table 1). There was a significant positive correlation between MDA and 4-HNE ($p = .002$). No correlation was found between FeNO and NOx or aldehydes, as well as between NOx and MDA or 4-HNE. In asthmatic children, 4-HNE values were lower in male than in female ($p = .02$) while no difference was found in levels of NOx, MDA or FeNO according to gender (Table 2). NOx levels were higher in children with allergic asthma ($p = .001$), especially in those with aeroallergen polysensitization, while 4-HNE levels were lower (Table 2). There was no association between SPT results and MDA values or FeNO (Table 2). Levels of aldehydes, NOx and FeNO were not associated with asthma control (Table 2) or with lung function. There was no difference between asthmatic children with $\text{FEV1/FVC} < 90\%$ ($n = 27$) and those with $\text{FEV1/FVC} > 90\%$ ($n = 6$) in FeNO (mean $\pm$ SEM [ppb] 14.80 ± 2.07 vs 12.25 ± 3.97 , $p = .54$), levels of NOx (mean $\pm$ SEM [μM] 7.02 ± 0.82 vs 6.16 ± 1.37 , $p = .50$), MDA (mean $\pm$ SEM [nM] 1.90 ± 0.21 vs 1.73 ± 0.49 , $p = .70$) and 4-HNE (mean $\pm$ SEM [nM] 0.37 ± 0.06 vs 0.39 ± 0.14 , $p = .99$). The reversibility test resulted positive in 14/33 asthmatics. There was no difference between children with bronchodilator reversibility and those without in levels of NOx (mean $\pm$ SEM [μM] 6.45 ± 0.83 vs 7.18 ± 1.08 , $p = .08$), MDA (mean $\pm$ SEM [nM] 1.84 ± 0.32 vs 1.89 ± 0.25 , $p = .62$), 4-HNE (mean $\pm$ SEM [nM] 0.41 ± 0.10 vs 0.35 ± 0.06 , $p = .13$) and FeNO (mean $\pm$ SEM [ppb] 15.39 ± 3.45 vs 13.56 ± 1.98 , $p = .11$).

TABLE 1. NOx and aldehydes (MDA, 4-HNE) in EBC from 33 subjects with asthma and 29 control subjects

Biomarkers in EBC	Children with asthma ($n = 33$)	Controls ($n = 29$)	p -value
NOx, μM	6.87 ± 0.71	3.87 ± 0.37	.001
MDA, nM	1.87 ± 0.19	2.28 ± 0.27	.21
4-HNE, nM	0.37 ± 0.05	0.61 ± 0.07	.006

Note

Data are shown as mean $\pm$ SEM.

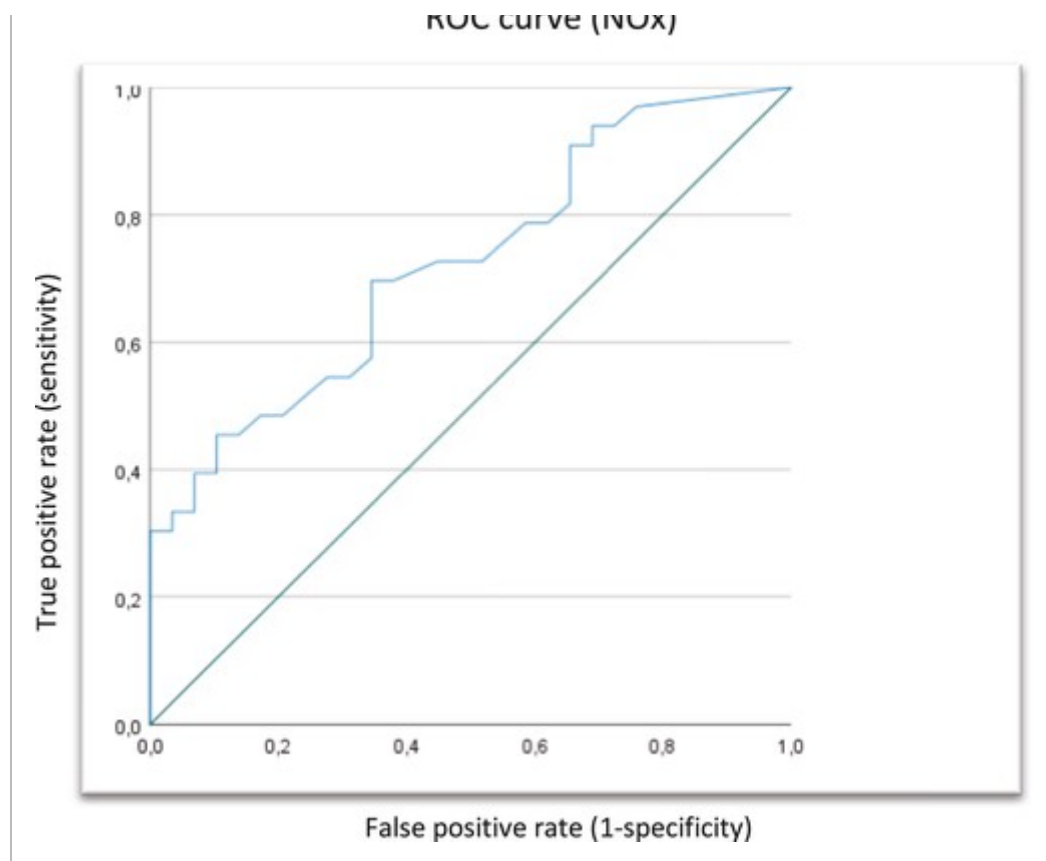


FIGURE 1

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ROC curve for the discriminating power of NOxLog_{10} (exhaled nitric oxide metabolites) between asthmatic and healthy subjects. AUC is 0.723 with an asymptotic confidence interval of 0.95% (lower limit 0.59 – upper limit 0.84)

TABLE 2. Characteristics of 33 asthmatic children, FeNO level and biomarker concentrations in EBC

Children with Asthma (<i>n</i> = 33)	NOx (μM)	MDA (nM)	4-HNE (nM)	FeNO (ppb)
Gender				
Female	5.16 ± 0.55	2.17 ± 0.20	0.64 ± 0.08	12.53 ± 2.46
Male	5.62 ± 0.63	2.00 ± 0.22	0.41 ± 0.05	14.92 ± 2.22
	(<i>p</i> = .6)	(<i>p</i> = .6)	(<i>p</i> = .02)	(<i>p</i> = .5)
SPT				
Positive	7.02 ± 0.75	1.84 ± 0.20	0.32 ± 0.07	14.82 ± 1.91
Negative	3.92 ± 0.35	2.28 ± 0.25	0.65 ± 0.04	9.72 ± 2.25
	(<i>p</i> = .001)	(<i>p</i> = .17)	(<i>p</i> = .000)	(<i>p</i> = .12)
SPT				

Children with Asthma (<i>n</i> = 33)	NOx (μM)	MDA (nM)	4-HNE (nM)	FeNO (ppb)
Poly-sensitization	6.89 ± 0.82	1,88 ± 0.22	0.33 ± 0.04	12.4 ± 1.61
Mono-sensitization	4.44 ± 0.45	2.19 ± 0.23	0.60 ± 0.06	19.3 ± 4.64
	(<i>p</i> = .01)	(<i>p</i> = .37)	(<i>p</i> = .005)	(<i>p</i> = .08)
Asthma control				
Controlled (C-ACT >19)	5.46 ± 0.50	1.99 ± 0.18	0.50 ± 0.05	12.07 ± 1.60

Note

Data are shown as mean ± SEM.

Our study found significantly higher EBC NOx levels and low EBC 4-HNE values in asthmatic children than in controls. In contrast, EBC MDA levels in children with asthma were similar to those of controls. To our knowledge, the simultaneous measurement of NOx and aldehydes in the EBC of asthmatic children has not been previously reported. Our findings are consistent with previous studies showing increased EBC NOx values in adolescent asthmatics,⁴ elevated EBC NO₂ concentration⁶ and total EBC NO₂/NO₃ levels in children with asthma. Yet, other studies found no association between levels of NO₂ and NO₃ in EBC and childhood asthma. Such discrepancies may be explained by differences in study populations, collection devices or sensitivity of the NOx analysis method. Our results extend previous observations in asthmatic adolescents,⁴ by showing an association between increased EBC NOx levels and aeroallergen poly-sensitization also in asthmatic children. This would reflect the condition of nitrosoactive stress that is typically described in eosinophilic inflammation. However, in agreement with previous investigations,⁷ we did not find a correlation between NOx and FeNO. This suggests that FeNO may reflect a milder degree of eosinophilic airway inflammation than NOx. Along this line, we showed significantly higher FeNO levels in children with poly-sensitization. FeNO values increased during exacerbation while EBC NO₂/NO₃ levels did not change.⁸ So, another explanation may be that in asthmatic subjects, the oxidative stress may be persistent and perhaps follow the inflammatory peak that increases FeNO. Regarding EBC aldehydes, we observed that 4-HNE levels were decreased, especially in male and in children with atopic asthma and inhalant poly-sensitization. Our findings are consistent with a previous small clinical study.⁵ We can speculate that low EBC 4-HNE concentrations are the consequence of its conjugation with glutathione that forms a more potent inducer of inflammation than non-conjugated HNE. Another hypothesis is that oxidative stress mechanisms might have depleted 4-HNE. Additional studies are needed to understand this point.

We did not find any difference between asthmatics and controls in EBC MDA concentrations.

Accordingly, higher MDA levels were reported in children with asthma exacerbation, but they did not differ between asthmatics and healthy subjects after a 5-day course of corticosteroids.⁵ In contrast, Dut et al⁹ showed higher levels of MDA in asthmatic children, 45% of them were atopic, than in controls. Since MDA concentrations might be related to a neutrophilic inflammatory response, a different origin of the oxidant stress may explain our divergent findings. In support of this, Dut et al⁹ studied a population in which asthmatics were atopic in 45% of cases, while in our population they were atopic in 94% of cases. Another explanation may be the different assay used for the measurement of MDA.

The strengths of our study are the prospective design with carefully diagnosed cases of asthma, defined EBC collection and assays and the measure of several markers. Possible limitations include relatively low participant number and lack of aeroallergen challenge.

In conclusion, biomarkers of oxidative stress are an important area, which needs to be investigated to understand its role in the pathophysiology of asthma. We found that both increased NOx and decreased 4-HNE in EBC may enable to distinguish children with asthma and with atopic asthma. This suggests that diagnostic cut-off points of these markers may have a practical implication in the management of asthmatic children.

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CONFLICT OF INTEREST

All authors declare no conflict of interests.

AUTHOR CONTRIBUTIONS

SR, MC and CC conceived and designed the study. SR, LP and FM managed the clinical study. AR and RC analysed EBC biomarkers. SR, MC, AR, LP and CC critically reviewed the data and wrote the manuscript. All Authors approved the final version of the manuscript submitted.

ETHICAL APPROVAL

The study was conducted in accordance with Helsinki Declaration as revised in 2013. Approval was obtained from the local ethics committee (no 73–2019, September 2019). Informed written consent of participants was obtained.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author [S.R.] upon reasonable request.

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