

PEDIATRIC ASTHMA

Factors influencing the levels of exhaled carbon monoxide in asthmatic childrenMilos Jesenak, MD, PhD¹, Peter Banovcin, MD, PhD¹, Zuzana Havlicekova, MD, PhD¹, Dusan Dobrota, MD, PhD², and Eva Babusikova, MSc, PhD²¹Department of Paediatrics and ²Department of Medical Biochemistry, Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava, Martin, Slovak Republic**Abstract**

Objective: Bronchial asthma is characterised by chronic airway inflammation commonly associated with increased oxidative stress. Exhaled carbon monoxide (eCO) levels could act as markers of both oxidative stress and allergic inflammation. We aimed to study eCO levels in asthmatics and detect the possible factors influencing them. **Methods:** We studied 241 asthmatic children and 75 healthy children. The differences in eCO levels among various asthmatic phenotypes and the correlations between eCO and other measured parameters (spirometric indices, Asthma Control Test score, exhaled nitric oxide, total IgE, blood eosinophils and marker of oxidative damage of proteins) were analysed. **Results:** Levels of eCO widely differed according to the selected characteristics of asthma. Asthmatics showed higher eCO concentrations than controls (1.44 ± 0.12 ppm vs. 0.91 ± 0.11 ppm, $p < 0.001$). Acute exacerbation of asthma was accompanied by a significant increase in eCO compared to the clinically controlled stage (2.17 ± 0.36 ppm vs. 1.33 ± 0.13 ppm, $p < 0.001$). Atopic, non-atopic asthma and asthma associated with allergic rhinitis (AR) showed elevated levels of eCO. The levels of eCO negatively correlated with the marker of protein oxidation in asthmatics, especially in atopic form and during acute exacerbation. **Conclusions:** In a population of asthmatic children, eCO levels could be considered as a marker of both allergic inflammation and oxidative stress in the airways. Concomitant AR and asthma control were the most important factors affecting the levels of eCO in asthmatic children. However, our results do not support the use of routine eCO in the clinical practice.

Keywords

Airway inflammation, asthmatic phenotype, bronchial asthma, oxidative stress, exhaled carbon monoxide

History

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Introduction

Bronchial asthma is one of the most common chronic respiratory diseases in children. The most characteristic pattern of this disease is continuous airway inflammation, which causes typical bronchial hyperreactivity and leads to irreversible airway re-modelling if not treated appropriately. Although significant advances have been made in the understanding and management of asthma in adults, less information and clear strategies are available for asthma management in children [1]. In addition to well-known triggers such as aeroallergens, food allergens, cold air, aspirin and viral infections, oxidative stress also plays an essential role in asthma development and persistence [2–4]. Recently, several inflammometric methods have become an important part of managing asthma (e.g. fractional exhaled nitric oxide [FENO] or some markers in exhaled breath condensate). These non-invasive and reproducible tools are of special value,

especially in children. Many studies have confirmed that the analysis of exhaled breath can be used for disease monitoring and the modification of the applied therapy. Besides the commonly used FENO measurement, several authors have suggested that exhaled carbon monoxide (eCO) can also be used in bronchial asthma [5–9] and in several other respiratory diseases such as allergic rhinitis (AR) [10], chronic pulmonary obstructive disease [11], bronchiectasis [12], cystic fibrosis [13], primary ciliary dyskinesia [12] or acute respiratory infections [10,14]. The eCO was studied also as a potential marker for evaluation of lifestyles and stress levels [15]. It has been proposed that eCO could be another non-invasive and useful marker of allergic inflammation and increased oxidative stress and can be used in the clinical management of bronchial asthma [2,5,7]. However, the utility of eCO as a marker of inflammation and its potential diagnostic value in the clinical practice has not been till today fully characterized and established [16]. Moreover, CO has been studied also as potential effective anti-inflammatory agent in preclinical animal studies with various chronic inflammatory diseases [17].

In a large group of children with bronchial asthma, we aimed to measure the levels of eCO in different phenotypes of

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childhood bronchial asthma (regarding the atopy, concomitant AR and achieved clinical control) and identify the possible associations between eCO levels and different factors and characteristics of asthmatic children.

Methods

Study population

We examined 316 schoolchildren (aged 12.61 ± 3.71 years, 55.7% boys). The study was performed at the Department of Paediatrics at the University Hospital Martin (Slovak Republic). Bronchial asthma was defined as recurrent airway obstruction manifested by wheezing and dyspnoea that was relieved spontaneously or by bronchodilator therapy (as defined in Global Initiative for Asthma). The diagnosis of bronchial asthma was based on the history of wheeze or cough during the last 12 months, use of any asthma medication in the last 12 months and a doctor's diagnosis of asthma ever. No tests of bronchial reversibility were used to support a diagnosis of asthma. Subjects were analysed according to the degree of asthma control (controlled – stable asthma vs. uncontrolled – acute asthma), atopy (atopic vs. non-atopic asthma) or concomitant AR (asthma only vs. asthma with AR). Children suffering from any serious internal diseases (immune, cardiovascular, gastrointestinal, neurological and respiratory) other than bronchial asthma were excluded from the study. The children suffering from acute respiratory infection or with the history of infection during the last four weeks were also excluded from the study. Altogether, we enrolled 241 asthmatic children, as well as 75 healthy control subjects of comparable age without any allergic disease and without subclinical atopy. All the regular asthma medications were continued unchanged. None of the subjects smoked, and the children exposed to passive smoking were excluded from the study. The children or their parents completed the standardised Asthma Control Test® (ACT; in two version according to the age of the tested subjects) [18].

Levels of eCO and FE_{NO} were measured in all children. The time between the measurement of eCO and FE_{NO} was at least 30 min. eCO was measured with Micro 4 Smokerlyzer® (Bedfont, Kent, UK), sensitive to 0–250 parts per million (ppm) of CO with a resolution of 1 ppm. The instrument was calibrated with a gaseous mixture containing 50 ppm CO in accordance with the manufacturer's instructions. Ambient CO levels in the testing room were measured before testing the subject. The tested subjects were asked to exhale fully, inhale deeply to full capacity and hold their breath for 15 s before exhaling slowly into a disposable Smokerlyzer® mouthpiece at a constant flow. This procedure gave the concentration of carbon monoxide in exhaled breath in ppm. The measurement was repeated twice with 1 min of normal breathing between each measurement, and the mean value was used for statistical analysis. During the measurement of both eCO and FE_{NO}, the classic nose clip was used to exclude the contribution of the sinuses. The levels of FE_{NO} were measured online according to the recommendations of the ERS/ATS standard [19], using a portable nitric oxide analyser (NIOX-MINO®, Aerocrine, Stockholm, Sweden). We also performed basic spirometry (KoKo DigiDoser-Spirometer; nSpire Health Inc., Louisville, CO) according

to the ATS/ERS recommendations [20]. The eCO and FE_{NO} values were estimated prior to the spirometric examination.

In all children, we performed skin prick tests (SPTs) with a panel of common inhalant allergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat dander, dog dander, mixed cereals, mixed grasses, mixed moulds and mixed trees; ALK-ABELLO, Hørsholm, Denmark). A SPT with allergens was defined as positive if the mean of the longest diameter and the diameter perpendicular to it and its mid-point was ≥ 3 mm. All the tests were performed by the same trained operator. Those children with at least one positive SPT were considered atopic.

The study was approved by the Ethics Committee of Jessenius Faculty of Medicine in Martin, Comenius University in Bratislava. An informed consent was obtained and signed by the parents of all the tested children.

Plasma protein sulphydryl groups

Plasma for determination of protein markers of invasive damage was prepared from blood by centrifugation for 20 min, $2000 \times$ at 4°C . The total concentration of reduced sulphydryl groups (–SH, proteins, oligopeptides, glutathione and amino acids) was determined by spectrophotometry [21]. Two millilitres of Ellman's reagent (0.250 mol/l TRIS-HCl, pH 8.2, 10 mmol/l 5,5'-dithiobis 2 nitrobenzoic acid) was added to 100 μl of sample and incubated for 15 min at room temperature. Absorbance was read at 412 nm and –SH group content was calculated using the molar absorption coefficient of $13\,600\text{M}^{-1}\text{cm}^{-1}$ after subtraction of blank absorbance from absorbance of the sample. A population of 75 healthy subjects was utilised to determine the normal value.

Statistical analysis

Data are expressed as means $\pm$ standard error of the mean unless otherwise indicated. Data were analysed with the software package SPSS version 11.0 (SPSS Inc., Chicago, IL). Student's two-tailed *t* test was used for comparison between two independent groups of normally distributed data and Mann–Whitney *U* test for non-normally distributed data. One-way ANOVA or Kruskal–Wallis tests were used for statistical comparison among more than two groups of subjects. Pearson correlation was used to calculate the relationships between the various measured variables (with Spearman correlation when indicated). *p* Values less than or equal to 0.05 were considered statistically significant.

Results

We examined 316 schoolchildren (aged 12.61 ± 3.71 years, 55.7% boys), consisting of 241 asthmatic children and 75 healthy controls of comparable age. The characteristics of the studied population are shown in Table 1. The asthmatic population was composed of 141 boys (58.5%) and 100 girls (41.5%). Regarding the clinical control for asthma, 29 children (12.0%) were examined during acute exacerbation (episode of progressive worsening of asthma symptoms such as shortness of breath, wheezing, chest tightness and/or coughing), while the rest of the children had good or fully clinically controlled asthma according to the results of ACT (88.0%, 212/241). In our study, 30.3% of asthmatics (73/241)

did not suffer from any other allergic co-morbidity, but 69.7% (168/241) also had the symptoms of concomitant AR. According to the results of the SPTs with the panel of common aeroallergens, 67.6% of the children suffered from so-called atopic asthma (163/241), and the rest was non-atopic (78/241, 32.4%).

The analysis of subjects according to the degree of asthma control (controlled – stable asthma vs. uncontrolled – acute asthma), atopy (atopic vs. non-atopic asthma) or concomitant AR (asthma only vs. asthma with AR) yielded different FE_{NO} values among the asthmatic subgroups and healthy children. The increase in FE_{NO} was observed during acute exacerbation, in atopic asthmatics and in asthmatic children with concomitant allergic rhinoconjunctivitis (Table 2). Spirometric indices were in general decreased in the asthmatic children compared to healthy subjects (FEV₁: 94.37 ± 0.99% predicted vs. 98.04 ± 1.54% predicted, $p=0.048$; MEF_{25–75%}: 88.95 ± 1.98% predicted vs. 97.92 ± 3.12% predicted, $p=0.017$). Children with asthma only (without AR), atopic asthma and during acute exacerbation showed decreased FEV₁ and MEF_{25–75%} in comparison with controls. Asthmatics with acute exacerbation had a lower total score of ACT than those who were clinically stable (Table 2).

Table 1. General characteristics of the studied population.

Parameter	Asthmatic subjects (N/%)	Healthy subjects (N/%)	<i>p</i>
Total number	241 (100)	75 (100)	–
Male	141 (58.5)	39 (52.0)	n.s.
Female	100 (41.5)	36 (48.0)	n.s.
Age [years]	12.54 ± 0.28	13.25 ± 0.22	n.s.
Age range [years]	6–18	6–18	n.s.
Atopy	163 (67.6)	–	–
Family history for asthma	92 (38.2)	6 (8.0)	$p<0.001$
Age of diagnosis of asthma	7.23 ± 3.19	–	–
Controlled (stable) asthma	212 (88.0)	–	–
Uncontrolled (acute) asthma	29 (12.0)	–	–
Total IgE [IU/mL]	234.40 ± 46.31	68.35 ± 21.98	$p<0.01$
Eosinophils [10 ⁹ /L]	0.38 ± 0.12	0.16 ± 0.11	$p<0.01$
Treatment			
ICS	82 (34.0)	–	–
LTRA	93 (38.6)	–	–
ICS+LABA	103 (42.7)	–	–
Antihistamines	182 (75.5)	–	–

ICS – inhaled corticosteroid, LABA – long-acting beta-2-agonist, LTRA – leukotriene receptor antagonist and n.s. – not significant.

Table 2. Comparison of the selected parameters in the subgroups of asthmatic children and healthy controls.

	FE _{NO} [ppb]	eCO [ppm]	ACT [score]	FEV ₁ [%predicted]	MEF _{25–75%} [%predicted]
Asthma (241)	25.98 ± 1.76 ^a	1.44 ± 0.12 ^a	22.63 ± 0.21	94.37 ± 0.99 ^b	88.95 ± 1.98 ^c
Acute asthma (29)	67.00 ± 8.01 ^{a,d}	2.17 ± 0.36 ^{a,d}	20.75 ± 0.77 ^d	93.00 ± 4.03	83.17 ± 5.31 ^b
Stable asthma (212)	20.60 ± 1.24 ^a	1.33 ± 0.13 ^b	22.88 ± 0.20	94.52 ± 1.02 ^b	89.58 ± 2.12 ^b
Atopic asthma (163)	34.14 ± 2.63 ^{c,d}	1.27 ± 0.14 ^b	22.52 ± 0.26	93.84 ± 1.26 ^b	86.88 ± 2.18 ^c
Non-atopic asthma (78)	12.81 ± 0.88	1.45 ± 0.19 ^c	23.00 ± 0.34	96.16 ± 1.75 ^b	92.28 ± 4.32
Asthma only (73)	21.26 ± 2.55 ^{c,e}	1.29 ± 0.18 ^{b,e}	22.76 ± 0.40	90.18 ± 0.67 ^{a,d}	82.63 ± 3.77 ^{c,f}
Asthma+allergic rhinitis (168)	28.19 ± 2.28 ^a	1.51 ± 0.15 ^c	22.59 ± 0.24	96.41 ± 1.19	92.11 ± 2.23
Controls (75)	12.57 ± 0.73	0.91 ± 0.11	–	98.04 ± 1.54	97.92 ± 3.12

FE_{NO} – fractional exhaled nitric oxide, eCO – exhaled carbon monoxide, ACT – Asthma Control Test, FEV₁ – forced expiratory volume in 1 s, and MEF_{25–75%} – mean expiratory flow.

Comparison between two asthmatic subgroups: ^d $p<0.001$, ^e $p<0.01$, ^f $p<0.05$.

Comparison between asthmatic subgroup and healthy controls: ^a $p<0.001$, ^b $p<0.05$, ^c $p<0.01$.

The levels of eCO were increased in asthmatics compared to healthy controls (1.44 ± 0.12 ppm vs. 0.91 ± 0.11 ppm, $p<0.001$). Comparing the various subgroups of asthma, children in acute exacerbations showed significantly elevated eCO concentration relative to those who were clinically controlled, and both subgroups had significantly higher eCO levels than healthy controls (Figure 1). Atopic and non-atopic asthmatics displayed similar eCO values, but differed significantly from healthy subjects (Table 2). Children with isolated asthma without nasal allergic symptoms as well as asthmatic subjects with concomitant AR yielded increased eCO amounts in comparison with the control group. Concomitant AR contributed to the increased levels of eCO compared to children with asthma only (Table 2).

Uncontrolled form of asthma (29 children) could modulate the final results in the whole asthmatic population. Therefore, we excluded children with acute form of asthma and repeated all analysis (FE_{NO}, eCO, FEV₁, MEF_{25–75%}), and we achieved similar results like in the whole asthmatic population. We observed only increased eCO concentrations in the exhaled breath of non-atopic asthmatics compared to healthy children (1.36 ± 0.19 ppm vs. 0.91 ± 0.11 ppm, $p=0.036$) and in asthmatics with concomitant AR in comparison with healthy controls (1.39 ± 0.17 ppm vs. 0.91 ± 0.11 ppm, $p=0.049$).

Levels of carbon monoxide in exhaled breath did not correlate with any spirometric indices, the scores obtained in the ACT[®], total IgE levels or eosinophils in peripheral blood. Exhaled CO concentrations were slightly positively correlated with the levels of FE_{NO} (Figure 2). There were no differences in eCO levels between the patients treated with leukotriene modifiers as monotherapy and the patients treated with inhaled corticosteroids without leukotriene modifiers (1.31 ± 0.75 ppm vs. 1.32 ± 0.27 ppm, $p>0.05$).

We found a significant correlation between the marker of oxidative protein damage and carbon monoxide in exhaled breath in asthmatic patients (eCO vs. –SH: $R=-0.232$, $p=0.001$). There was a significant mild to moderate negative correlation between the concentration of –SH groups and eCO in atopic asthmatics (eCO vs. –SH: $R=-0.232$, $p=0.001$, Figure 3) and during acute exacerbation of asthma (eCO vs. –SH: $R=-0.174$, $p=0.027$). This correlation was borderline in clinically controlled asthma, while among non-atopic asthmatics and in control group, we did not find these correlations. The higher the oxidative damage is, the lower –SH groups concentration is detected as a result of

Figure 1. The differences in the levels of exhaled carbon monoxide among asthmatics with acute exacerbations, stable clinically controlled asthmatics and healthy control children.

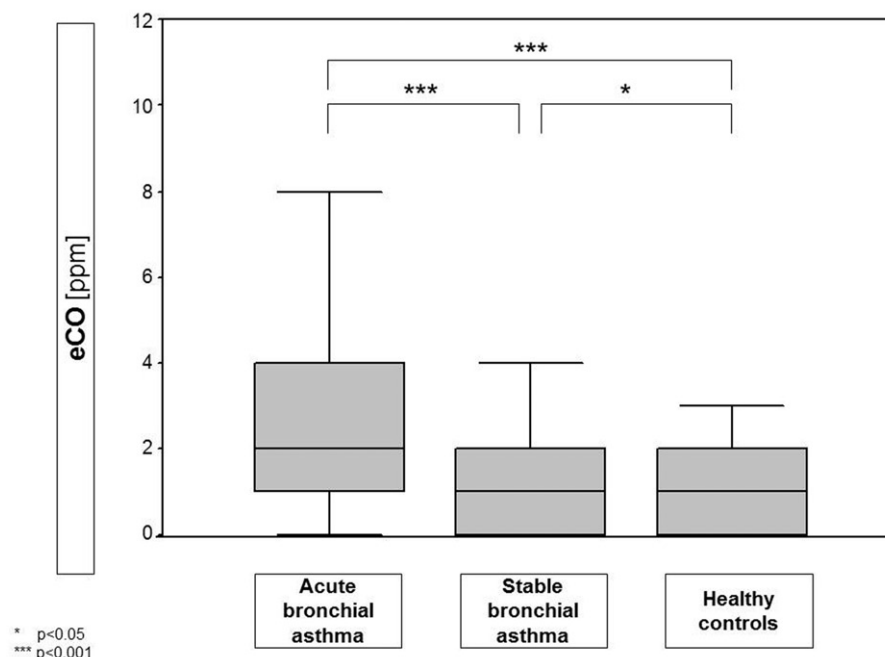
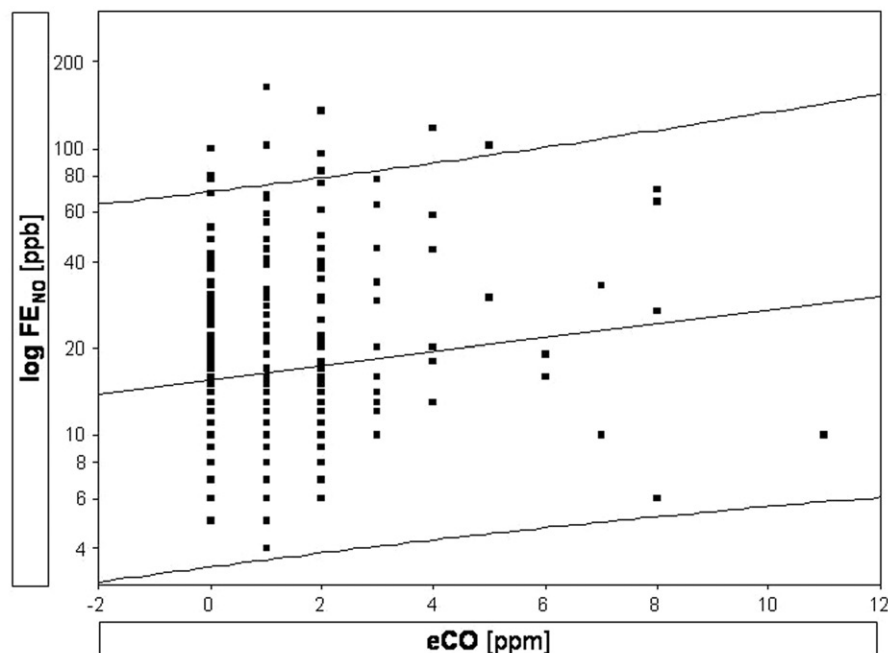


Figure 2. Correlation between the values of exhaled carbon monoxide (eCO) and fractional exhaled nitric oxide (FENO) ($y = 1.794x + 21.027$, $R = 0.128$, $p = 0.040$).



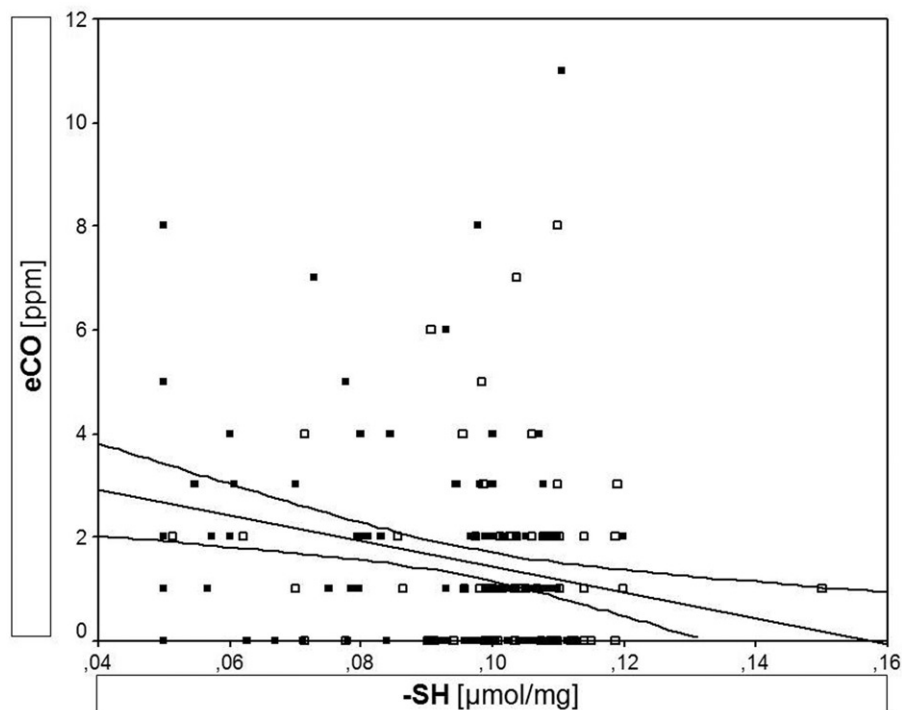
the oxidation of free –SH groups. The increased oxidative stress is accompanied by increased production of eCO.

Discussion

In our study, we analysed changes in the carbon monoxide concentration in exhaled air in a population of asthmatic and healthy schoolchildren. The measured values of eCO in asthmatics and healthy, non-asthmatic children observed in our study were similar [7,8,10] or lower to those of previous studies [22–24]. We found that the children suffering from bronchial asthma gave higher eCO readings than healthy controls. Increased eCO levels in asthmatics may reflect the expression and activation of heme-oxygenase-1 in the

airways, which is activated by inflammation and oxidative stress [2]. There are conflicting results about the differences in eCO levels between asthmatics and healthy subjects. Similar to our results, other authors also observed an elevation in eCO amounts in asthmatics compared to healthy controls [7,25]. Conversely, in other studies, asthmatics showed nearly the same levels of eCO as healthy subjects [9,26–28]. Recently, in a population of adults with bronchial hyperreactivity, eCO concentrations did not show any changes to serve as a biomarker for asthma activity [29]. However, we were able to show some differences in this parameter according to disease activity and control. It has previously been observed that children and adult asthmatics differ in the levels of eCO [29].

Figure 3. Negative correlation between the values of exhaled carbon monoxide (eCO) and concentration of free sulphydryl groups (–SH) in a group of atopic asthmatics ($y = -24.877x + 7.672$, $R = -0.232$, $p = 0.001$).



In our study, children during acute exacerbation showed significantly increased eCO concentration compared to either stable asthmatics or healthy controls. Another similar study with 20 participants demonstrated a significant increase in eCO during asthma exacerbation [27]. Ohara et al. [6] found that eCO levels in infrequent episodic asthmatic children were not different from those in healthy schoolchildren, but increased significantly during asthma attacks. Several authors have suggested that the elevation of eCO amounts could be related to the severity or clinical control of bronchial asthma. Yamaya et al. [27] observed that the concentration of eCO in unstable severe asthmatics was significantly higher than that in stable severe asthmatics. Children with persistent asthma showed higher eCO values compared to those with infrequent episodic asthma and healthy children. Rises in eCO concentrations were also observed in acute lower respiratory tract infections, which can lead to the asthma exacerbation [30]. Conversely, Kong et al. [31] did not confirm differences among acute asthmatics, stable asthmatics and controls.

In our study, children with atopic and non-atopic asthma had similar eCO levels, which were significantly higher in comparison with healthy subjects. Moreover, we found a significant negative correlation between eCO and marker of protein oxidation. Therefore, it could be suggested that the eCO reflects the oxidative stress observed in chronic airway diseases rather than the allergic inflammation itself, which is better expressed by the levels of FE_{NO} . Increased levels of eCO may be an early and non-invasive marker of airway inflammation in asymptomatic atopic subjects, regardless of the presence of bronchial asthma, but its sensitivity is lower in comparison with FE_{NO} [32]. The finding of several authors that eCO is increased equally in different inflammatory airway diseases including asthma [2,28], bronchiectasis [12] and cystic fibrosis [32] suggests that CO production has a profound link with inflammatory

processes, regardless of their atopic or non-atopic nature [12]. In addition, there are several other factors that influence the final concentration of eCO, such as holding breath, upper or lower respiratory infections [9,14,26], corticosteroid treatment [13,32], antibiotic treatment [30], non-systemic inflammatory response and passive or active smoking [2,12,33].

Children suffering from concomitant AR showed increased levels of eCO compared to those with isolated bronchial asthma and healthy controls. Gratziou et al. [34] found this difference for exhaled nitric oxide, but not for eCO. Studying the changes of eCO levels in patients with AR during and out of pollen season, Monma et al. [35] noted that eCO concentration was significantly increased in symptomatic patients compared to non-symptomatic ones. It can be suggested that the increase in eCO levels in patients with AR may be produced from both the lower and upper airways [29,36]. Shaoging et al. [37] did not find significant differences in eCO levels between AR and the control group.

In our study, we found a significant slightly positive correlation between the concentrations of eCO and FE_{NO} , which was also observed by Rosias et al. [22], but not by the others [23,25]. Interestingly, in contrast to FE_{NO} , eCO did not show any expiratory flow dependency [38]. We did not find any correlation between eCO and lung function parameters or ACT scores, consistent with the other studies [8,9,11,26,28,31]. Conversely, in the study of Yamaya et al. [27], there was a significant relationship between exhaled CO concentrations and FEV_1 in all adult asthmatic patients. It is possible that eCO could be correlated to some spirometric parameters, but the data in the literature are generally inconsistent [7,12,32]. The correlation between eCO and spirometric indices could be due to persistent airway inflammation (detected by increased eCO), which leads to airway remodelling and a subsequent decline in lung functions [27]. It is evident that eCO is less related to lung

function parameters and symptoms than FE_{NO} . eCO increases during the early and late asthmatic reactions independently of the changes in airway calibre, while exhaled nitric oxide rises only during the late reaction and follows the increase in carbon monoxide and fall in the FEV_1 with time. A clear elevation in the eCO amounts observed in patients with asthma during the late and early response immediately after the allergen challenge [7] was not confirmed by another similar study [26].

The measurement of eCO levels involves a cheap, reproducible and practical method with the use of a portable CO analyser. There are no circadian variations in eCO concentrations [27], as can be seen in the measurement of FE_{NO} [39]. Furthermore, there are no seasonal changes in mean exhaled CO and in contrast to FE_{NO} , eCO levels are not related to the changes in airway calibre [14,23]. The measurement of eCO may be useful in several respiratory chronic diseases with more intensive airway inflammation and increased oxidative stress (bronchiectasis, cystic fibrosis and primary ciliary dyskinesia) [12,13,33]. In these diseases, eCO could be an even more sensitive and representative marker of oxidative stress than FE_{NO} [13]. It can also be supposed that the measurement of eCO may not be specific for some special respiratory diseases or pathology [30]. Non-invasive measurement of exhaled CO may provide complementary data for assessing asthma control in children; however, elevated eCO levels are non-specific and could be related to an acute viral illness, which is a common trigger for acute asthmatic exacerbations [9].

Conclusions and key findings

In conclusion, more studies are required to define the biological role of CO in the airway inflammation of bronchial asthma, and its utility in clinical practice should be revised. A consensus on the proper technique for eCO measurement in children and adults is still missing and should be established. Although the differences and changes in eCO levels are not as expressive as those of FE_{NO} , the measurement of eCO can be used in asthma research, but its clinical utility as a marker of asthma severity or allergic inflammation is probably lower than that of FE_{NO} . Current guidelines do not recommend the use of eCO in the management of bronchial asthma. Although eCO might not prove to be a clinically useful marker of asthmatic inflammation, its measurement has helped to understand the role of heme oxygenase in allergic and non-allergic airway diseases [40]. In our population of asthmatic children, eCO levels increased with the loss of asthma control and were associated with AR. Our data enlarged the results of recent meta-analysis, which concluded that eCO was elevated in asthmatics. Our results showed that eCO levels reflect disease severity and control and associated allergic comorbidities [41].

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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