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EIA and GC/MS analysis of 8-isoprostane in EBC of children with problematic asthma

S. Carraro*, P.E. Cogo*, I. Isak*, M. Simonato*, M. Corradi#, V.P. Carnielli¶ and E. Baraldi*

ABSTRACT: Asthmatic airways are characterised by enhanced oxidative stress, which can be studied by measuring biomarkers, such as 8-isoprostane. The aims of the present study were: 1) to measure the concentrations of 8-isoprostane in exhaled breath condensate (EBC) and urine of children with problematic and well-controlled asthma; 2) to compare the concentrations of 8-isoprostane measured by gas chromatographic/negative ion chemical ionisation mass spectrometry (GC/NICI-MS) and by an enzymatic immunoassay (EIA).

We recruited 20 asthmatic allergic children, 13 with well-controlled asthma and seven with problematic asthma. They underwent exhaled nitric oxide measurements and spirometry, and both EBC and urine samples were collected. 8-isoprostane was measured in EBC by GC/NICI-MS and EIA.

8-isoprostane concentrations in EBC were significantly higher in children with problematic asthma than in children with well-controlled asthma ($p=0.01$). An acceptable reproducibility emerged between GC/NICI-MS and EIA (coefficient of reproducibility 11.5 $pg \cdot mL^{-1}$). 8-isoprostane levels measured in urine did not correlate with those measured in EBC.

We showed that 8-isoprostane in EBC was significantly increased in children with problematic asthma, suggesting a role for oxidative stress in this asthma phenotype. In addition we found an acceptable reproducibility of EIA compared to GC/NICI-MS, even if the latter method had higher accuracy.

KEYWORDS: Children, exhaled breath condensate, 8-isoprostane, mass spectrometry, problematic asthma, urine

A number of recent studies have demonstrated an enhanced oxidative stress in asthma, as a consequence of an increased release of oxidant species and a decline in antioxidant defences [1]. It is difficult to measure reactive oxygen species (ROS) directly because they are highly reactive and short-lived, so oxidative stress is often studied by measuring products of the interaction between ROS and lipids, proteins or DNA [1]. Isoprostanes are prostaglandin-like compounds formed from the free radical catalysed peroxidation of arachidonic acid, a mechanism independent of the cyclooxygenase [2, 3]. 8-isoprostane is, therefore, a stable product of lipid peroxidation and it is a reliable marker of oxidative stress [4]. This marker can be measured in exhaled breath condensate (EBC), a biofluid collected noninvasively by cooling exhaled air during tidal breathing [5]. EBC is a promising methodology inasmuch as the condensate composition is believed to reflect that of the airway lining fluid. However, the EBC technique has not been fully standardised yet and there are several methodological pitfalls, such as the sensitivity of the available assays, and unresolved issues, such as the definition of the exact anatomic origin of the biomarkers measured [5].

Increased levels of 8-isoprostane have been found in EBC of asthmatic subjects, confirming the role of oxidative stress in the pathogenesis of asthma [6, 7].

In many studies, an immunoenzymatic technique (enzymatic immunoassay; EIA) has been used to measure 8-isoprostane in EBC [7–10]. Though the EIA approach is known to have some weaknesses in the analysis of EBC (inasmuch as the assay works close to its detection limit), to our knowledge no published studies have compared EIA with a reference analytical method based on mass spectrometry in the study of EBC.

It is widely acknowledged that asthma can be easily controlled in most cases, although there is a small subgroup of children with recurrent

AFFILIATIONS
*Dept of Paediatrics, Unit of Respiratory Medicine and Allergy, University of Padova, Padova, #Dept of Clinical Medicine, Nephrology and Health Sciences, University of Parma, Parma, and ¶Division of Neonatology, Dept of Neonatal Medicine, Salesi Children's University Hospital, Ancona, Italy.

CORRESPONDENCE
S. Carraro
Dept of Paediatrics, Respiratory Medicine and Allergy Unit
University of Padova
Via Giustiniani 3
35128 Padova
Italy
E-mail: silvia.carraro.1@unipd.it

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severe exacerbations or chronic symptoms despite prescription of multiple drugs. The term ''problematic asthma'' has recently been proposed to describe these children [11] and efforts should be made to better characterise this phenotype.

The aims of the present study were 1) to measure the concentrations of 8-isoprostane in EBC of children with problematic and well-controlled asthma; and 2) to compare the concentrations of 8-isoprostane measured by gas chromatographic/negative ion chemical ionisation mass spectrometry (GC/NICI-MS) and by EIA. In addition, we compared the 8-isoprostane measured in EBC and in a urine sample, collected at the same time, in order to establish whether EBC, being a biofluid collected directly from the lung, can reflect pathological processes in the lung better than a systemic matrix, such as urine.

METHODS

Patients

20 asthmatic children were enrolled, whose asthma was diagnosed by a paediatric respiratory physician based on their clinical history (cough, shortness of breath, recurrent wheezing and chest tightness) and an increase in forced expiratory volume in 1 s (FEV_1) after salbutamol (400 μg) >12%, according to international guidelines [12]. Seven of these children had problematic asthma [11] while the other 13 children had well-controlled asthma [12]. Problematic asthma was defined as the presence of chronic symptoms and/or frequent exacerbations and/or persistent airflow obstruction, despite treatment with high dose of inhaled steroids (ICS) [12] combined with long-acting β_2-agonists (n=7), montelukast (n=2) and theophylline (n=1). Cystic fibrosis, immunodeficiency and chronic rhinosinusitis had been excluded. Gastro-oesophageal reflux had been demonstrated and treated in three of these children. Nine of the 13 children with well-controlled asthma were treated with low–medium doses of inhaled steroids and seven were also taking long-acting β_2-agonists.

All asthmatic children were atopic, sensitised to at least one airborne allergen, as demonstrated by skin-prick tests.

At recruitment, children underwent physical examination, exhaled nitric oxide fraction (F_{ENO}) measurement and spirometry. Patients were instructed to abstain from short-acting bronchodilators for 8 h and from long-acting bronchodilators for 18 h before spirometry.

EBC and urine samples were collected and stored at -80°C, and subsequently analysed by EIA (for EBC and urine) and GC/NICI-MS (for EBC alone). In eight children, two EBC samples were collected 1 h apart to evaluate the analytical reliability.

All the study procedures were conducted in the afternoon.

EBC and urine analyses were performed at the Dept of Paediatrics of the University of Padova, Padova Italy.

The ethics committee at our hospital reviewed and approved the protocol and all parents gave their informed consent.

F_{ENO} and pulmonary function measurement

F_{ENO} was measured using the NIOX system (Aerocrine, Stockholm, Sweden), following the European Respiratory Society (ERS)/American Thoracic Society (ATS) guidelines for measuring F_{ENO} in children [13]. Children inhaled nitric oxide-free air to total lung capacity and exhaled through a dynamic flow restrictor with a target flow of 50 $mL \cdot s^{-1}$ for ⩾6–7 s. No nose clip was used. The NIOX system was calibrated using a 200 ppb nitric oxide tank (Lindegas Hoek Loos Speciality Gases, Amsterdam, the Netherlands), according to the manufacturer's instructions.

Lung function was measured by means of a 10-L bell spirometer (Biomedin, Padova, Italy) and the best of three manoeuvres was expressed as a percentage of predicted reference values, according to ERS/ATS guidelines [14].

EBC collection

EBC was collected and processed according to recent ATS/ERS recommendations [5], using the TURBO-DECCS (a transportable unit for research on biomarkers obtained from disposable exhaled condensate collection systems) (Medivac, Parma, Italy).

As reported elsewhere [15], the TURBO is a refrigerating system that relies on a thermoelectric module producing a Peltier effect. The cold side of the Peltier module is connected to an aluminium support shaped to house the test tube [16]. The temperature is maintained constant during the collection (we used a collecting temperature of -4°C). The TURBO is supplied with the DECCS, a disposable respiratory system that consists of a mouthpiece equipped with a one-way valve and a reliable saliva trap, connected to a collecting vial (50 mL) by means of a tube [16]. The children breathed tidally through the mouth for 15 min, while sitting comfortably and wearing a nose clip. They kept their mouth dry during EBC collection by periodically swallowing excess saliva. EBC samples were stored at -80°C in polypropylene tubes until assay.

Urine collection

Urine samples, collected right after the EBC collection, were immediately stored at -80°C in polypropylene tubes until assay.

EIA of 8-isoprostane

One 50-μL aliquot of unextracted EBC was assayed in duplicate according to the manufacturer's protocol for the 8-isoprostane (8-iso-$PGF_{2\alpha}$)-specific EIA kit (Cayman Chemical, Ann Arbor, MI, USA) and 8-iso-$PGF_{2\alpha}$ concentrations in EBC were measured by plotting the values identified in the sample with the 8-iso-$PGF_{2\alpha}$ standard calibration curve (3.9–500 $pg \cdot mL^{-1}$).

8-iso-$PGF_{2\alpha}$ concentrations in urine were measured using the same EIA kit. Samples were prepared prior to the assay using a purification protocol recommended by the manufacturer [17]. Briefly, the pH of the urine was adjusted to ~4.0 with 0.1 M HCl and a 0.5 mL aliquot of urine was extracted in duplicate on a SPE C-18 cartridge (Alltech, Casalecchio di Reno, Italy), previously rinsed with 5 mL of methanol followed by 5 mL of UltraPure water (water purified with a Milli Q device (Millipore, Vimodrone, Italy)). The columns were then washed with 5 mL of UltraPure water, 5 mL of hexane and allowed to dry. The 8-iso-$PGF_{2\alpha}$ was eluted with 5 mL ethyl acetate containing 1% methanol. The eluate underwent silica gel chromatography carried out with a solution of chloroform/

methanol/acetic acid/water (80:18:1:0.8 v/v). 8-iso-$PGF_{2\alpha}$ was eluted with 4 mL ethanol, dried at 37°C under a nitrogen stream and reconstituted with EIA buffer. The antiserum used in the EIA has 100% cross-reactivity with 8-isoprostane and 20.6%, 4.00%, 1.84% and 1.70% respectively with $PGF_{3\alpha}$, 2,3-dinor-$PGF_{2\alpha}$, $PGE_{2\alpha}$ and 2,3-dinor-$PGF_{1\alpha}$ PGE_1, as declared by the manufacturer. The lowest detection limit of the assay was 3.9 pg·mL^{-1}. 8-iso-$PGF_{2\alpha}$ concentrations were expressed in pg·mL^{-1} for EBC and in ng·mmol^{-1} creatinine for urine samples. Creatinine urine concentrations were measured with the SPOTCHEM II Creatinine Reagent Strip in the SPOTCHEM Analyser (A. Menarini Diagnostics, Firenze, Italy).

GC/NICI-MS of 8-isoprostane

8-iso-$PGF_{2\alpha}$ concentrations in EBC were measured using a GC/NICI-MS approach with a stable isotope dilution method modified from that described by MILNE *et al.* [18], using a quadrupole mass spectrometer (Voyager; Thermoquest, Rodano, Italy). Sample preparation was carried out prior to assaying, first by extraction and then by derivatisation. After acidifying 0.5-mL aliquots of EBC, in duplicate, to pH 3 with HCl 0.1 M, 2 ng of the deuterated internal standard 8-iso-$PGF_{2\alpha}$-d_4 (Cayman Chemical) was added. After adding the internal standard, the mixture was vortexed and applied on an HLB (Oasis Waters, Vimodrone, Italy) cartridge previously prepared by rinsing with 2 mL of methanol followed by 2 mL of UltraPure water (acidified to pH 3.0 with HCl) and left to dry. The columns were washed with 3 mL of UltraPure water (acidified to pH 3.0 with HCl) followed by 3 mL of hexane. 8-iso-$PGF_{2\alpha}$ was eluted with 4 mL of ethyl acetate and dried at 40°C under a nitrogen stream. The extract was then converted into pentafluorobenzyl ester by treating it with a mixture of 40 μL of 10% pentafluorobenzyl bromide in acetonitrile and 20 μL of 10% *N,N*-di-isopropyl ethylamine in acetonitrile at room temperature for 30 min. The reagents were dried under a nitrogen stream, then the 8-iso-$PGF_{2\alpha}$ was converted into trimethylsilyl ether derivative by adding 20 μL of *N*-methyl-*N*-(tert-butyldimethylsilyl)-trifluoroacetamide and 10 μL of pyridine; the mixture was incubated at 40°C for 20 min, dried under a nitrogen stream and the derivatised 8-iso-$PGF_{2\alpha}$ was redissolved in 20 μL of undecane for analysis by GC/NICI-MS. 8-iso-$PGF_{2\alpha}$ was separated on a 30 m × 0.25 mm × 0.25 μm ULTRA2 fused silica capillary column (J&W Scientific Agilent Technologies Italia SpA, Cernusco sul Naviglio, Italy). The oven temperature was programmed for 1 min at 190°C, 20°C·min^{-1} from 190°C to 300°C, and maintained at 300°C for 12 min. Methane was used as carrier gas for the GC/NICI-MS. The ion monitored for 8-iso-$PGF_{2\alpha}$ was *m/z* 569, while for the internal standard it was *m/z* 573.

Statistical analysis

Normally distributed data were recorded as mean ± SEM. Non-normally distributed data (urinary 8-isoprostane and F_{ENO} values) were reported as median and interquartile range and were log-transformed (achieving a normal distribution) to perform the statistical analysis.

The between-method reproducibility of the 8-isoprostane measurements using EIA and GC/NICI-MS was assessed by Bland–Altman analysis [19]. The coefficient of reproducibility was calculated as 1.96 × SD of the differences between the measurements performed with the two methods, and it was used to define the limits of agreement.

The reliability of EIA and GC/NICI-MS was evaluated by calculating the intraclass correlation coefficient (ICC) in a subgroup of children who performed two consecutive EBC collections 1 h apart.

The comparison between children with problematic asthma and children with well-controlled asthma was performed by unpaired t-test for 8-isoprostane EBC concentrations and log-transformed urinary 8-isoprostane concentrations and F_{ENO} values.

Correlations were evaluated by applying Pearson's test. Results were considered significant at a value of $p<0.05$.

Power calculation revealed that a sample size of seven enables a difference in EBC 8-isoprostane levels of 30 pg·mL^{-1} to be detected with a power of 83% at a two-sided α level of 0.05.

RESULTS

8-isoprostane in children with problematic and well-controlled asthma

We found that children with problematic asthma had significantly higher 8-isoprostane levels than children with well-controlled asthma. This was true for the measurements obtained by both GC/NICI-MS ($p=0.008$) (fig. 1) and EIA ($p=0.01$) (table 1).

On the contrary, the comparison of urine 8-isoprostane levels did not show any difference between problematic and well-controlled asthma ($p=0.4$) (table 1).

F_{ENO} was no different in problematic and well-controlled asthmatic children ($p=0.32$) (table 1).

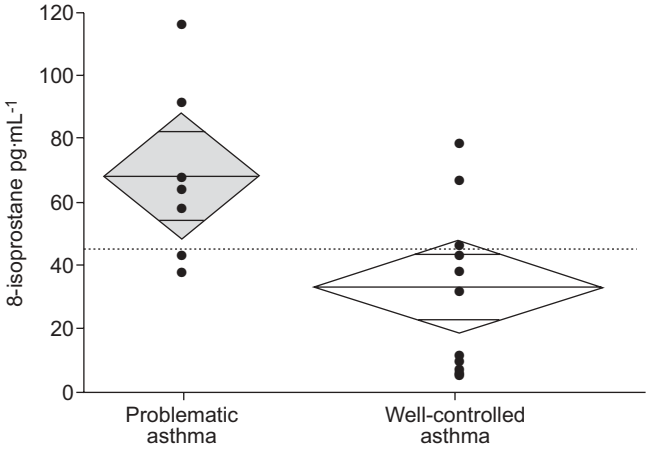

FIGURE 1. The diamond plot represents exhaled breath condensate 8-isoprostane levels in children with problematic asthma and with well-controlled asthma measured by gas chromatographic/negative ion chemical ionisation mass spectrometry. The line through the centre of each diamond represents the group mean. The top and bottom vertices are the upper and lower 95% confidence limits. The horizontal dotted line is the overall mean. The short horizontal lines flanking the means are overlap marks. The black dots are the individual values. The difference between the two groups is statistically significant ($p=0.008$).

TABLE 1 Anthropometric characteristics, 8-isoprostane concentrations, spirometric parameters and exhaled nitric oxide values in children with problematic and well-controlled asthma

	Problematic asthma	Well-controlled asthma	p-value
Subjects (males) n	7 (4)	13 (7)	
Age (range) yrs	9.6 (6–13)	11.1 (6–15)	
Height cm	140.6±6.3	146.8±3.1	
Weight kg	35.6±3.9	45.8±3.6	
8-isoprostane in EBC by GC/NICI-MS pg·mL^{-1}	68.0±10.3	32.8±6.6	0.008
8-isoprostane in EBC by EIA pg·mL^{-1}	74.0±12.5	35.3±7.4	0.01
8-isoprostane in urine EIA ng·mmol^{-1} creatinine	35.4 (30.6–60.6)	50.6 (35.1–74.9)	0.44
FVC % pred	89±6	96±3	0.26
FEV_1 % pred	76±5	89±3	0.048
$FEF_{25–75\%}$ % pred	59±7	82±7	0.05
FEV_1/FVC %	79±4	84±2	0.20
F_{ENO} ppb	16 (9.5–47.5)	27 (17.3–48.0)	0.36

Data are expressed as mean±SEM and median (interquartile range), unless otherwise stated. EBC: exhaled breath condensate; GC/NICI-MS: gas chromatographic/negative ion chemical ionisation mass spectrometry; EIA: enzymatic immunoassay; FVC: forced vital capacity; % pred: % predicted; FEV_1: forced expiratory volume in 1 s; $FEF_{25–75\%}$: forced expiratory flow between 25% and 75% of FVC; F_{ENO}: exhaled nitric oxide fraction.

When either the whole group of children or the well-controlled group was considered, no difference was found between those treated with ICS and those steroid naive (p=0.63 and p=0.59, respectively).

Reproducibility of EBC 8-isoprostane measurements

The Bland–Altman plot showed an acceptable reproducibility of the 8-isoprostane measurements obtained by GC/NICI-MS and EIA (fig. 2). The coefficient of reproducibility was 11.5 pg·mL^{-1} and the 95% limits of agreement were -15.4 pg·mL^{-1} and +7.7 pg·mL^{-1}, with all the values falling within this range.

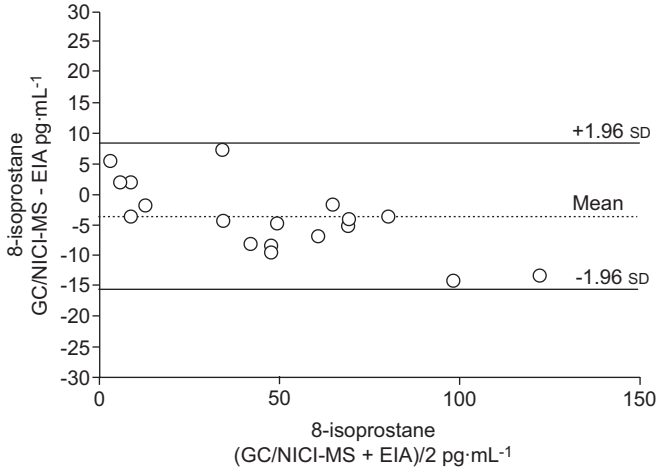

FIGURE 2. Bland–Altman plot of exhaled breath condensate 8-isoprostane measurements obtained with gas chromatographic/negative ion chemical ionisation mass spectrometry and enzymatic immunoassay (coefficient of reproducibility 11.5 pg·mL^{-1}). In the y-axis are reported the differences and in the x-axis the means of the measurements performed with the two methods. The coefficient of reproducibility is 1.96×SD (standard deviation of the differences).

Reliability

A subgroup of children provided two EBC samples 1 h apart. The ICC for these pairs of samples was 0.72 for GC/NICI-MS (n=8) and 0.53 for EIA (n=5).

Correlations of EBC 8-isoprostane with lung function and F_{ENO} measures

The measures of 8-isoprostane performed by means of EIA show a negative correlation with forced expiratory flow between 25% and 75% of forced vital capacity ($FEF_{25–75\%}$) and with FEV1/forced vital capacity (FVC) close to statistical significance (p=0.05, r= -0.4 and p=0.06, r= -0.4 respectively). When the EBC measurements obtained by GC/NICI-MS were considered, the correlations with $FEF_{25–75\%}$ and FEV1/FVC were both statistically significant (p=0.03, r= -0.5).

No correlation emerged between the 8-isoprostane levels measured by EIA and FVC (p=0.67) or FEV_1 (p=0.12), or between the 8-isoprostane measured by GC/NICI-MS and FVC (p=0.66) or FEV_1 (p=0.08).

No correlation was found between EBC 8-isoprostane levels and F_{ENO}.

The measurements obtained in urine samples showed no correlation with the spirometric parameters or F_{ENO} levels.

Correlation between measurements in urine and EBC

No correlation emerged between the 8-isoprostane concentrations in urine and in EBC, measured either by GC/NICI-MS (p=0.27) or by EIA (p=0.23).

DISCUSSION

The present study showed that EBC 8-isoprostane, a marker of oxidative stress, was significantly higher in children with problematic asthma than in children whose asthma was well controlled (fig. 1). 8-isoprostane was measured with both a reference analytical method and EIA: we found an acceptable

reproducibility (fig. 2), although GC/NICI mass spectrometry afforded greater accuracy.

We also found a significant negative correlation between 8-isoprostane and both $FEF_{25-75\%}$ and FEV_1/FVC, which are sensitive indicators of airway obstruction and show a declining gradient in children with increasingly severe asthma [20]. 8-isoprostane is a stable product of free-radical catalysed arachidonic acid peroxidation independent of the cyclo-oxygenase, and it is a recognised marker of oxidative stress [4]. Oxidative stress can cause airway narrowing, both by directly damaging the airway epithelium due to lipid peroxidation and because of the bronchoconstriction induced by the release of arachidonic acid [21, 22]. There is also evidence of a receptor-mediated contraction of airway smooth muscle caused by isoprostane [4]. Our data confirm a close link between increased oxidative stress and airflow limitation in asthmatic children. This finding is in keeping with previous data reported by our own [7] and other groups [6, 10, 23], supporting the existence of a correlation between oxidative stress and asthma severity. In a recent study, FITZPATRICK *et al.* [24] demonstrated an imbalance between oxidants and anti-oxidants, with reduced GSH (reduced glutathione) and increased GSSG (oxidised glutathione) levels in bronchoalveolar lavage of children with severe refractory asthma, supporting a key role for oxidative stress in the pathogenesis of severe asthma.

In our study, $F_{E}NO$ levels did not differ between problematic and well-controlled asthma cases, nor did they correlate with 8-isoprostane EBC levels. $F_{E}NO$ is considered a biomarker of eosinophilic airway inflammation [25] and its levels can predict response to ICS [26]. Our findings confirm that $F_{E}NO$ and 8-isoprostane reflect different aspects of the pathogenic mechanisms behind asthma. Taken together, our data suggest that children with problematic asthma may have an inflammatory phenotype in which oxidative stress plays a central role. The resulting oxidative damage may lead to a persistent airway obstruction and poor response to steroid therapy. In keeping with our results, previous studies have already reported that EBC 8-isoprostane levels are poorly affected by ICS therapy [6–8]. A potential role for anti-oxidant treatments in asthma management has recently been proposed [1] and measuring 8-isoprostane might be used in the future to identify asthmatic children likely to benefit more from the use of such new therapeutic approaches.

From a methodological standpoint, in the present study we compared the measurements of 8-isoprostane (8-iso-$PGF_{2\alpha}$) in the EBC of asthmatic children using two different methods, *i.e.* GC/NICI-MS (a reference analytical method) and an EIA assay that is cheaper and easier to perform but less sensitive and specific [5].

When the Bland–Altman plot was used to compare the measurements obtained with the two methods, we found a coefficient of reproducibility of 11.5 $pg \cdot mL^{-1}$, with all values falling within the limits of agreement (fig. 2), showing that the reproducibility between the two methods is acceptable. The weaknesses of the EIA technique should be borne in mind nonetheless. In fact, when we calculated the ICC, we found that the reliability of EIA was not as good as that of GC/NICI-MS (ICC 0.53 and 0.72, respectively). In addition, the visual inspection of the Bland–Altman plot (fig. 2) shows that the differences between the measurements obtained with the two methods were negatively skewed, suggesting that EIA tends to overestimate the 8-isoprostane concentrations in EBC. This may be due to the cross-reactivity of the EIA antibody with structurally related 8-iso-$PGF_{2\alpha}$ isomers as reported in the method section and also by other authors [27, 28]. Neither of these isomers coelutes with the 8-iso-$PGF_{2\alpha}$ used to measure 8-isoprostane by GC/NICI-MS, as amply explained by MILNE *et al.* [18].

The advantages of GC/NICI-MS over commercially-available immunoassay kits include the high sensitivity and specificity of the mass spectrometric approach [29], which yields quantitative results in the low picogramme range; its drawbacks are that it is labour-intensive and requires considerable outlays for equipment by comparison with the low cost and relative ease of use of immunoassay kits. Furthermore the sensitivity and specificity of the immunoassay kits vary considerably from one manufacturer to another. More recently, new methods for 8-iso-$PGF_{2\alpha}$ analysis, based on liquid chromatographic mass spectrometry (LC/MS), have been developed, offering the advantage of a simpler sample preparation than in GC/MS because no derivatisation of the molecule is required [30]. Despite major advances in the sensitivity of LC/MS instrumentation, one concern with these assays relates to the detection limits in biological fluids, which are often higher than those using GC/MS [30, 31]. For these reasons we can infer that the GC/MS analysis can still be considered the reference analytical method.

In this study, a urine sample was collected immediately after collecting the EBC and subsequently analysed for the presence of 8-isoprostane. To our knowledge, no previous studies on asthmatic subjects have measured the same biomarker in both urine (a systemic matrix) and EBC, which is a biological fluid reflecting airway lining fluid composition [5]. Though EBC collection is easy and entirely noninvasive, it is time-consuming and younger children may have trouble cooperating for long enough to complete the procedure, whereas urine samples are very quick and easy to collect. These observations provided the rationale for simultaneously analysing urine and EBC samples with a view to ascertaining whether urine can reflect the inflammatory processes in the asthmatic lung as accurately as EBC does. Our results demonstrate that urinary 8-isoprostane levels do not correlate with those measured in EBC, nor do they show any correlation with lung function parameters or with the asthma phenotype. Urine samples are likely to be affected by the metabolism of the whole body and our data suggest that EBC is by far superior for studying lung inflammation and oxidative stress.

The present study has a number of limitations. Children with problematic asthma are only a minority of the asthmatic children and the small sample size prevents us from comparing the two sub-phenotypes of problematic asthma described by BUSH *et al.* [32] (*i.e.* difficult-to-treat asthma and severe therapy resistant asthma). As previously suggested [11], there is a need for multicentre studies enabling the recruitment of a sufficient number of patients to allow for these aspects to be investigated.

We also recognise the methodological limits of the EBC technique [5]. As regards 8-isoprostane, it has been demonstrated that different condenser coatings lead to different percentage of recovery in EBC [9]. This is why the results of studies applying different condensers cannot be readily compared. Moreover, from an analytical point of view, interferences due to matrix effect are possible when 8-isoprostane is measured by EIA, and they are favoured by the very low concentration of detected substances [33].

In conclusion, our study demonstrated that EBC 8-isoprostane levels are higher in children with problematic asthma, suggesting a role for airway oxidative stress in this asthma phenotype. In addition, we found an acceptable reproducibility of EIA compared with GC/NICI-MS, even if the latter method had higher accuracy.

STATEMENT OF INTEREST

Statements of interest for S. Carraro, V.P. Carnielli and E. Baraldi can be found at www.erj.ersjournals.com/misc/statements.dtl

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