Oxidative stress biomarkers and asthma characteristics in adults of the EGEA study

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ABSTRACT Asthma is an oxidative stress related disease, but associations with asthma outcomes are poorly studied in adults. We aimed to study the associations between several biomarkers related to oxidative stress and various asthma outcomes.

Cross-sectional analyses were conducted in 1388 adults (mean age 43 years, 44% with asthma) from the Epidemiological Study of the Genetics and Environment of Asthma (EGEA2). Three blood antioxidant enzyme activities (biomarkers of response to oxidative stress) and exhaled breath condensate 8-isoprostanes and plasma fluorescent oxidation products (FlOPs) levels (two biomarkers of damage) were measured. Associations between biomarkers and 1) ever asthma and 2) asthma attacks, asthma control and lung function in participants with asthma were evaluated using regression models adjusted for age, sex and smoking.

Biomarkers of response were unrelated to asthma outcomes. Higher 8-isoprostane levels were significantly associated with ever asthma (odds ratio for one interquartile range increase 1.28 (95% CI 1.06–1.67). Among participants with asthma, 8-isoprostane levels were negatively associated with adult-onset asthma (0.63, 0.41–0.97) and FlOPs levels were positively associated with asthma attacks (1.33, 1.07–1.65), poor asthma control (1.30, 1.02–1.66) and poor lung function (1.34, 1.04–1.74).

Our results suggest that 8-isoprostanes are involved in childhood-onset asthma and FlOPs are linked to asthma expression.

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Introduction

Asthma is a chronic airway inflammatory disease affecting ~350 million people worldwide [1]. This heterogeneous disease [2, 3] is now studied at cellular and molecular levels, offering new opportunities for its prevention and control [4, 5]. Oxidative stress, which reflects an imbalance between increased exposure to reactive oxygen species (ROS) and antioxidant defence, is involved in the pathophysiological mechanism of asthma [6]. In asthma, ROS are produced endogenously by metabolic reactions [7, 8] and exogenously by environmental factors (*e.g.* air pollutants and smoking) [8, 9].

To neutralise the overproduction of ROS, the organism develops antioxidant defences through an enzymatic system including superoxide dismutase (SOD) coupled to catalase and glutathione peroxidase (GPX) [10, 11]. These antioxidant enzymes, which are the first line of defence against the ROS, are biomarkers of interest in the response to oxidative stress [8]. In addition, ROS interact with lipids of cells membranes, especially arachidonic acid, releasing 8-isoprostanes, the end-products of lipid peroxidation [12]. However, few studies are available on the associations between biomarkers of response (SOD, GPX and catalase) [13–16] or biomarkers of damage (8-isoprostanes) [6, 12] and asthma characteristics particularly in adults, and their results are discordant.

Levels of fluorescent oxidation products (FlOPs), a global biomarker of oxidation processes, including protein and DNA oxidation and lipid peroxidation [17], are of growing interest in epidemiology. This biomarker of damage has been found to be associated with chronic diseases [18] such as coronary heart disease [19] and chronic kidney disease [17]. Nevertheless, to our knowledge, no studies have been conducted to evaluate the association between FlOPs and asthma.

Taking advantage of the extensive biological and phenotypic characterisation of >1000 adults in the Epidemiological Study of the Genetics and Environment of Asthma (EGEA) study, we aimed to investigate the associations between biomarkers of response and damage related to oxidative stress, measured from different biological compartments, and asthma outcomes.

Methods

Population and study design

EGEA is a French cohort study with three surveys over 20 years. The first EGEA survey (EGEA1) included cases with asthma, recruited in five chest clinics, their first-degree relatives and population-based controls, recruited in the early 1990s in five French cities (n=2047). A follow-up of the participants was completed in 2003–2007 (EGEA2), including 1601 subjects with complete examination, almost exclusively adults. At each survey, all subjects responded to a questionnaire based on international standardised tools to diagnose asthma and to determine respiratory and allergic symptoms, treatments and environmental exposures. The protocol and descriptive characteristics have been described previously [20, 21]. The EGEA collection is certified ISO 9001 and referenced in the Biobank network [22].

The present analyses used data collected at EGEA2. Only adult participants (aged ⩾16 years) with available data on measurement of any biomarker and smoking status were included (n=1388; online supplementary figure E1). Among adults, participants not included in the analyses (n=183) were similar to those included regarding age, sex, smoking status, body mass index (BMI) and asthma characteristics (online supplementary table E1).

Asthma outcomes

Asthma cases were participants who had positive responses to four questions from the validated and standardised British Medical Research Council, European Coal and Steel Community, American Thoracic Society (ATS) and European Community Respiratory Health Survey questionnaires: “Have you ever had attacks of breathlessness at rest with wheezing?”, “Have you ever had asthma attacks?”, “Was this diagnosis confirmed by a physician?” and “Have you had an asthma attack in the last 12 months?”, or a positive response to at least two questions and a positive review of medical records. Asthma in first-degree relatives of cases was defined as a positive answer to at least one of the first two questions [21, 23]. Among the 614

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participants with ever asthma included in the present analysis, 536 (87.3%) had a diagnosis of asthma confirmed by a physician. Among participants with ever asthma, current asthma was defined by the report of respiratory symptoms (wheeze, nocturnal chest tightness and attacks of breathlessness following strenuous activity, at rest or at night-time) or asthma attacks or use of inhaled and/or oral medicines because of breathing problems in the past 12 months [24]. Asthma control was assessed over a 3-month period as previously described [25], matching as closely as possible the Global Initiative for Asthma 2015 definition, based on frequency of daytime/night-time symptoms, use of reliever medication and activity limitations. Asthma exacerbations were defined by hospital or emergency admissions because of respiratory problems or use of oral steroids for breathing difficulties in the past 12 months [26].

More details on the definition of asthma outcomes, lung function, medication use and allergic and inflammatory characteristics are provided in online supplementary material.

Biomarkers related to oxidative stress

Biomarkers of response

Erythrocyte antioxidant enzyme activities were measured as previously described [27, 28] according to standardised procedures. The enzymatic activity was expressed in $U \cdot g^{-1}$ of haemoglobin (Hb) for SOD and GPX and in $k \cdot g^{-1}$ Hb for catalase (1k corresponds to the rate constant of the first-order reaction). All samples were analysed in duplicate or triplicate at the laboratory of biochemistry molecular biology of CHRU de Lille (Lille, France) according to validated and standardised procedures. The coefficient of variation was <10% for each enzymatic assay.

Biomarkers of damage

Exhaled breath condensate (EBC) was collected using an RTube according a standardised method as previously described [22]. Briefly, the RTube was rinsed with deionised water and dried thoroughly. Participants breathed orally at tidal volumes into a mouthpiece attached to a cold condenser (−20°C). They were seated comfortably with a headrest. All headrests and seat backs were tilted slightly to avoid any saliva contamination during breathing manoeuvres. Breathing was quiet and regular. After 15 min, EBC was immediately separated in aliquots and stored at −80°C according to standardised procedures.

8-isoprostane levels were measured in EBC using a specific enzyme immunoassay kit (8-isoprostanes EIA kit; Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's protocol. 50 µL of unextracted EBC was assayed in duplicate and the 8-isoprostane levels were calculated from a calibration curve obtained from the eight calibration points (0.8–2.0–5.1–12.8–32–80–200–500 $pg \cdot mL^{-1}$, where 0.8 $pg \cdot mL^{-1}$ is the lowest point). The lower limit of detection for 8-isoprostanes was 4.0 $pg \cdot mL^{-1}$ and the intra-assay coefficient of variation was <20%.

Plasma FlOP levels were measured as previously described [18, 29]. Briefly, plasma was extracted into a mixture of ethanol/ether (3/1 v/v) and measured using a spectrofluorimeter (360 nm excitation wavelength, 430 nm emission wavelength). Fluorescence was expressed as a unit of relative fluorescence intensity $(RFU) \cdot mL^{-1}$ of plasma.

Interplate variability, storage time and diurnal variation did not affect any of the biomarker levels (data not shown). More details on blood and EBC collection, as well as measurement of biomarkers are provided in the online supplementary material.

Statistical analyses

SOD, GPX, FlOPs and 8-isoprostanes were log-transformed due to their skewed distribution and expressed as geometric mean (Q1–Q3).

Associations between each biomarker and age, sex, BMI and smoking status were studied in participants without asthma, in order to evaluate these associations independently of the disease.

We used regression models to investigate the associations between each biomarker and ever asthma in all participants and between each biomarker and asthma characteristics, pulmonary function, allergic and inflammatory characteristics in participants with ever asthma. "Partly controlled" and "uncontrolled" asthma were regrouped into one class, due to the small number of asthmatics in the uncontrolled group.

Regression analyses were conducted using generalised estimated equations to take into account familial dependence between individuals. Multiple regression models considered age (continuous), sex and smoking status (never-, ex- or current smokers) as potential confounders. A sensitivity analysis on the association between FlOP levels and asthma outcomes was performed with adjustment for heavy smoking expressed as number of cigarette packs per year or daily tobacco consumption $(g \cdot day^{-1})$ instead of smoking status.

To facilitate interpretation of the results, we rescaled the biomarker levels using interquartile range, defined as the distance between the 25th and 75th percentiles, and compared participants with a typical "high" level of biomarker to participants with a typical "low" level.

Statistical analyses were performed using SAS statistical software (version 9.3; SAS Institute, Cary, NC, USA). A p-value of <0.05 was considered statistically significant.

Results

Characteristics of the participants

Table 1 shows the characteristics of the 1388 participants included in the analyses. Among them, 614 had ever asthma. Their mean age was 43 years, 51% were female and 23% were smokers. Participants with ever asthma were more often male (p=0.01) and younger than participants without asthma (p<0.0001). In addition, they had higher rates of allergic sensitisation, lower forced expiratory volume in 1 s (FEV_1) and higher bronchial hyperresponsiveness than participants without asthma (all p<0.0001).

Among participants with ever asthma, 89% had current asthma; 32% had partly controlled asthma and 11% had uncontrolled asthma; 34% had an age of asthma onset ⩾16 years; in the past 12 months, 66% reported use of any asthma treatments (inhaled or oral medicines), 40% reported use of inhaled corticosteroids (ICS), 38% had asthma attacks, 14% reported having had asthma exacerbations and 78% reported having had respiratory symptoms.

In all participants, the geometric means (Q1–Q3) of biomarkers of oxidative stress were 1218 (1036–1449) $U \cdot g^{-1}$ Hb for SOD and 39.1 (34.2–45.3) $U \cdot g^{-1}$ Hb for GPX; mean±SD of catalase was 163±40.4 $k \cdot g^{-1}$ Hb. The geometric means of 8-isoprostanes were 2.88 (1.31–6.66) $pg \cdot mL^{-1}$ and 93.3 (79.9–106) $RFU \cdot mL^{-1}$ for FlOPs.

Description of biomarkers of oxidative stress in participants without asthma

Among biomarkers of response, catalase activity was higher among those with higher BMI (p_{trend}=0.02) and was higher in current and ex-smokers than in nonsmokers (p_{trend}=0.004). However, no significant association was observed between catalase and heavy smoking. GPX activity was positively associated with age (p_{trend}=0.01) and was higher in females than in males (p<0.0001). SOD activity was negatively associated with age (p_{trend}<0.0001) and positively associated with BMI (p_{trend}=0.003) (online supplementary table E2).

Among biomarkers of damage, 8-isoprostane levels were negatively associated with age (p_{trend}=0.01), and was higher in females than in males (p=0.04). FlOPs levels were positively associated with age (p_{trend}<0.0001) and were higher in current and ex-smokers than in nonsmokers (p_{trend}<0.0001) (online supplementary table E3). Furthermore, in current and ex-smokers, FlOPs levels were positively associated with the number of packs of cigarettes per year (p_{trend}=0.03); and in current smokers, FlOPs levels were positively associated with daily tobacco consumption (p_{trend}=0.001) (online supplementary figure E2).

Associations between biomarkers and asthma status

The distribution of each biomarker according to ever asthma is shown in figures 1 and 2. There was no significant difference in SOD, GPX or catalase activities according to ever asthma. In contrast, 8-isoprostane levels were higher and FlOPs levels were lower in participants with ever asthma compared to those without asthma. After adjustment for age, sex and smoking status, only the association between 8-isoprostane levels and ever asthma remained significant (table 2).

Associations between biomarkers and asthma outcomes among participants with ever asthma

Among participants with ever asthma, there was no significant association between SOD, GPX and catalase activities and asthma outcomes or lung function (online supplementary table E4).

In contrast, biomarkers of damage were associated with several asthma outcomes (table 2). Lower levels of 8-isoprostanes were significantly associated with adult-onset asthma compared to childhood-onset asthma. No other significant associations were found.

Higher FlOPs levels were significantly associated with adult-onset asthma, poor asthma control, asthma attacks, any asthma treatment and use of inhaled corticosteroids in the past 12 months. Furthermore, a significant positive association was observed between FlOPs levels and poor lung function. Consistently, FlOPs levels was negatively correlated with FEV_1 (r=−0.16, p=0.0001). All these associations remained significant after adjustment for age, sex and smoking status, except for the association between FlOPs and adult-onset asthma. No significant association was found between FlOPs levels and respiratory symptoms (table 2). Results were unchanged when we adjusted for age, sex and heavy smoking expressed as number of pack-years or daily tobacco consumption (data not shown).

Among participants with ever asthma, no significant association was observed between any biomarkers and bronchial hyperresponsiveness.

TABLE 1 Description of participant characteristics, according to ever asthma

	All participants	Without ever asthma	With ever asthma	p-value[#]
Subjects n	1388	774	614	
Female	51.1	54.4	47.1	0.01
Age	43.1±16.5	46.2±15.9	39.1±16.3	<0.0001
Body mass index kg·m^{-2}				0.09
<20	10.6	9.33	12.2	
20–25	51.6	50.5	53.0	
25–30	27.8	30.1	24.8	
⩾30	10.0	10.1	9.93	
Subjects n	1365	761	604	
Smoking status				0.10
Non-smoker	50.1	50.1	50.2	
Ex-smoker	27.4	29.2	25.1	
Current smoker	22.5	20.7	24.8	
Age of asthma onset				
<4 years	30.5		30.5	
4–16 years	35.1		35.1	
⩾16 years	34.4		34.4	
Subjects n	573		573	
Current asthma	88.8		88.8	
Subjects n	572		572	
Asthma control				
Controlled	57.9		57.9	
Partly controlled	31.5		31.5	
Uncontrolled	10.6		10.6	
Subjects n	537		537	
Asthma attacks (past 12 months)	38.1		38.1	
Subjects n	611		611	
Exacerbation (past 12 months)	13.6		13.6	
Subjects n	573		573	
Respiratory symptoms (past 12 months)	54.6	36.2	77.8	<0.0001
Subjects n	1377	768	609	
Use of any asthma treatments[¶] (past 12 months)	34.8	10.3	65.6	<0.0001
Subjects n	1388	774	614	
Use of ICS (past 12 months)	19.6	3.24	40.4	<0.0001
Subjects n	1381	772	609	
FEV_1 % predicted	102±18.0	107±16.5	96.9±18.4	<0.0001
Subjects n	1360	757	603	
FEV_1 <80% predicted	9.7	5.42	15.1	<0.0001
Subjects n	1360	757	603	
Methacholine challenge[+] PD_{20} ⩽4 mg	44.3	26.9	67.6	<0.0001
Subjects n	851	487	364	
Skin prick test positivity[§]	56.3	38.5	78.8	<0.0001
Subjects n	1281	716	565	
Total IgE ⩾100 IU·mL^{-1}	43.0	29.5	60.1	<0.0001
Subjects n	1383	772	611	
Eosinophils count ⩾250 cells·mm^{-3}	26.9	17.2	39.2	<0.0001
Neutrophils count ⩾5000 cells·mm^{-3}	20.0	19.2	21.0	0.42

Data are presented as % or mean±SD, unless otherwise stated. ICS: inhaled corticosteroids only or in combination with long-acting β_2-agonist; FEV_1: forced expiratory volume in 1 s; PD_{20}: provocative dose causing a 20% fall in FEV_1; Ig: immunoglobulin. [#]: p-value of Chi-squared test for categorical variables and t-test for quantitative variables; [¶]: asthma treatments were defined by the use of inhaled and/or oral medicines in the past 12 months because of breathing problems; [+]: methacholine challenge test was not performed if baseline FEV_1 <80% predicted; [§]: skin prick test positivity was defined by a mean wheal diameter ⩾3 mm larger than the negative control for at least one of 12 aeroallergens.

Associations between biomarkers of damage, allergic sensitisation and markers of inflammation

In participants with ever asthma, we also studied the association between biomarkers of damage and allergic sensitisation markers (total immunoglobulin (Ig)E or skin prick test positivity), and did not observe any significant association (table 3).

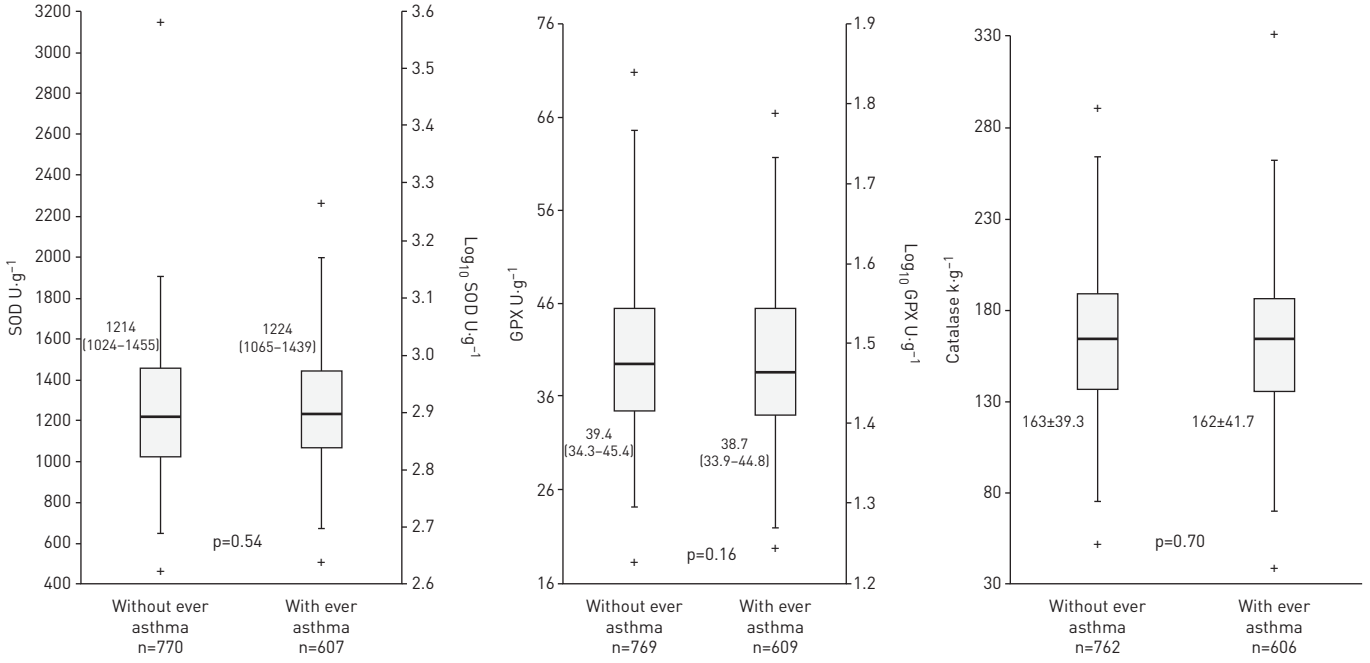

FIGURE 1 Levels of biomarkers of response related to oxidative stress according to ever asthma. Boxplots show the median (bar), the first and third quartiles (box), the 1st and 99th percentiles (whiskers) and the minimum and maximum (+) of the biomarkers level for each asthma status. The y-axis to the left represents the raw biomarker concentration and the y-axis to the right represents the $\log_{10}$ concentration. Catalase was not log-transformed. Data are presented as geometric mean (Q1–Q3) or mean±SD. SOD: superoxide dismutase; GPX: glutathione peroxidase.

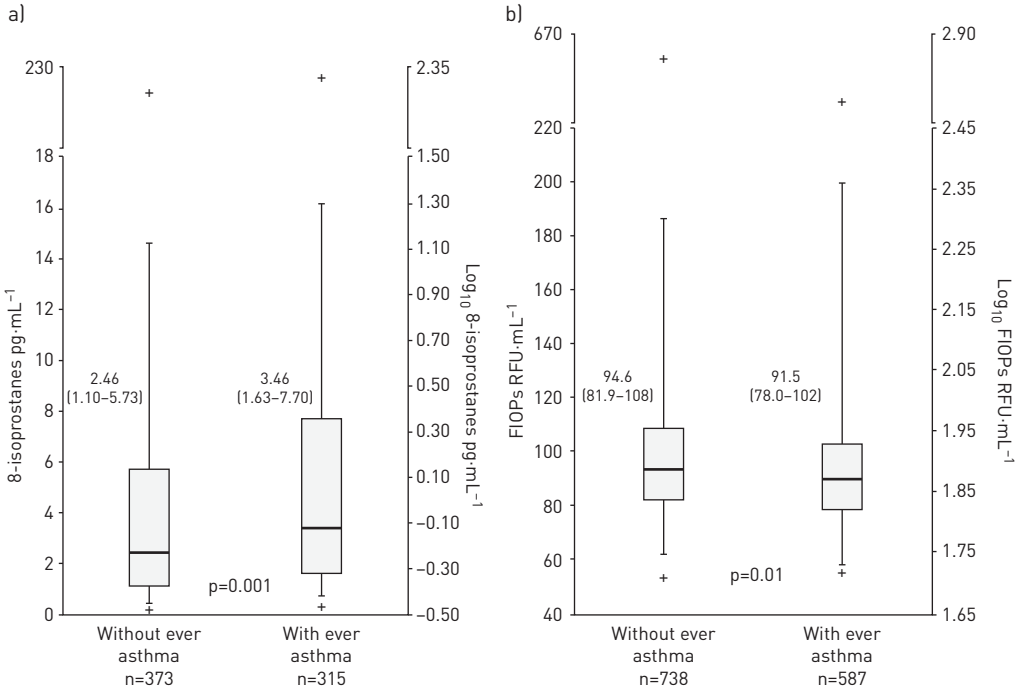

FIGURE 2 Levels of biomarkers of damage related to oxidative stress according to ever asthma. Boxplots show the median (bar), the first and third quartiles (box), the 10th and 90th percentiles (a), 1st and 99th percentiles (whiskers) (b) and the minimum and maximum (+) of the biomarker levels for each asthma status. FlOPs: fluorescent oxidation products. Data are presented as geometric mean (Q1–Q3).

TABLE 2 Associations between biomarkers of damage related to oxidative stress and various asthma outcomes

	8-isoprostanes			Fluorescent oxidation products		
	Subjects n	OR (95% CI)[#]	aOR (95% CI)[#]	Subjects n	OR (95% CI)[#]	aOR (95% CI)[#]
Asthma ever (*versus* never asthma)	688	1.40 (1.17–1.67)	1.28 (1.06–1.55)	1325	0.84 (0.73–0.96)	0.98 (0.86–1.13)
Among participants with ever asthma						
Adult-onset asthma[¶] (*versus* childhood-onset)	294	0.51 (0.35–0.76)	0.63 (0.41–0.97)	548	1.55 (1.25–1.93)	1.08 (0.85–1.38)
Current asthma (*versus* without current asthma)	295	0.65 (0.38–1.13)	0.69 (0.40–1.18)	547	1.44 (0.97–2.16)	1.38 (0.92–2.06)
Poor asthma control[+] (*versus* controlled asthma)	282	0.82 (0.59–1.15)	0.86 (0.61–1.22)	514	1.40 (1.11–1.77)	1.30 (1.02–1.66)
Asthma attacks in past 12 months	315	0.80 (0.58–1.10)	0.80 (0.57–1.12)	584	1.27 (1.04–1.55)	1.33 (1.07–1.65)
Exacerbation in past 12 months	297	0.75 (0.47–1.20)	0.79 (0.50–1.25)	549	1.18 (0.90–1.56)	1.11 (0.81–1.52)
Respiratory symptoms in past 12 months	311	0.73 (0.35–1.10)	0.76 (0.39–1.13)	582	1.17 (0.87–1.46)	1.11 (0.81–1.41)
Any asthma treatments in past 12 months[§]	315	0.77 (0.45–1.09)	0.80 (0.47–1.13)	587	1.34 (1.12–1.56)	1.29 (1.05–1.52)
Use of ICS in past 12 months	313	0.72 (0.51–1.00)	0.80 (0.57–1.11)	582	1.45 (1.19–1.77)	1.30 (1.05–1.60)
FEV_1 <80% predicted[+]	314	0.85 (0.52–1.39)	1.01 (0.64–1.60)	579	1.63 (1.28–2.07)	1.34 (1.04–1.74)
Methacholine challenge PD_{20} ⩽4 mg[+,f]	193	0.92 (0.54–1.30)	0.84 (0.42–1.26)	354	0.97 (0.68–1.26)	0.97 (0.65–1.28)

aOR: adjusted odds ratio; ICS: inhaled corticosteroids only or in combination with long-acting β_2-agonist; FEV_1: forced expiratory volume in 1 s; PD_{20}: provocative dose causing a 20% fall in FEV_1. [#]: OR expressed for an increase corresponding to the value of the interquartile range (distance between the 25th and 75th percentile) of each biological marker; adjusted for age, sex and smoking status; [¶]: defined as an age of asthma onset ⩾16 years and childhood-onset was the reference group; [+]: results were unchanged when restricting analyses to participants with current asthma; [§]: asthma treatments were defined by the use of inhaled and/or oral medicines in the past 12 months because of breathing problems; [f]: methacholine challenge test was not performed if baseline FEV_1 <80% predicted.

In addition, we studied the association between biomarkers of damage and blood neutrophil and eosinophil counts (table 3). No significant associations were observed between any biomarkers and eosinophil counts. However, a positive significant association was observed between FlOPs levels and high neutrophil counts (⩾5000 *versus* <5000 cells·mm^{-3}). We observed a consistent positive correlation between FlOPs and neutrophil count (r=0.12, p=0.005).

Discussion

The present study investigated the associations between biomarkers related to oxidative stress and various asthma outcomes. We found no significant association between any biomarkers of response and asthma outcomes. Regarding biomarkers of damage, a positive and significant association was observed between 8-isoprostane levels and ever asthma. Among participants with ever asthma, we showed for the first time a significant and positive association between 8-isoprostanes and childhood-onset asthma, while significant positive associations were shown between FlOPs levels and poor asthma control, asthma attacks, any asthma treatments, poor lung function and neutrophilic asthma.

The main strength of our study was the investigation of the association between several biomarkers involved in either the response to oxidative stress or related to damage caused by it, and various asthma characteristics. Indeed, while biomarkers of response are intracellular and reflect activities of antioxidant enzymes [8], they are present at the beginning of the "oxidative stress chain" [30, 31] in the continuum between environment and the disease. Conversely, FlOPs are a global marker of oxidative stress, reflecting a mixture of oxidation products from DNA, proteins and lipids [18], and 8-isoprostanes are a specific products of lipid peroxidation [32]. These two biomarkers of damage are present at the end of the oxidative stress chain and are more likely to be associated with asthma. Furthermore, we studied oxidative stress at the systemic level and close to the lung in exhaled breath condensate. Most of the participants with asthma were recruited in chest clinics as asthma cases, with a careful procedure set up to include true asthmatics using standardised and validated questionnaires. Others were recruited as first-degree relatives of asthmatic cases, based on answers to questions upon asthma diagnosis. This leads to a group of asthmatics with wide range of severity and response to methacholine. The detailed phenotypic characterisation included various asthma outcomes, which have been rarely studied in relation to oxidative stress biomarkers. No follow-up bias related to the asthma status and asthma-related phenotypes was shown in the EGEA study, and the adult asthmatics included in the present study are representative of the original study population of asthmatic cases and their first-degree relatives with asthma. Oxidant/antioxidant status, and thus biomarker levels, depend on the ability of each individual to counter oxidative reactions involving genetic factors, lifestyle and environmental factors. Although not all these parameters were taken into account in our analysis and unmeasured confounding can never be ruled out, we have

TABLE 3 Associations between biomarkers of damage related to oxidative stress, markers of allergy and markers of inflammation in participants with ever asthma

	8-isoprostanes			Fluorescent oxidation products		
	Subjects n	OR (95% CI)[#]	aOR (95% CI)[#]	Subjects n	OR (95% CI)[#]	aOR (95% CI)[#]
Markers of allergy						
Total IgE ⩾100 IU·mL^{-1}	314	0.98 (0.72–1.34)	0.85 (0.61–1.18)	584	0.99 (0.82–1.21)	1.17 (0.93–1.47)
Positive SPT	297	0.92 (0.62–1.37)	0.70 (0.47–1.05)	546	0.86 (0.68–1.08)	1.19 (0.90–1.57)
Markers of inflammation						
Neutrophils[¶] ⩾5000 cells·mm^{-3}	315	0.88 (0.62–1.26)	0.94 (0.65–1.36)	587	1.38 (1.11–1.72)	1.34 (1.06–1.70)
Eosinophils[¶] ⩾250 cells·mm^{-3}	315	0.87 (0.63–1.19)	0.84 (0.60–1.16)	587	0.94 (0.78–1.13)	1.02 (0.84–1.25)

aOR: adjusted odds ratio; SPT: skin prick test; Ig: immunoglobulin. [#]: OR expressed for an increase corresponding to the value of the interquartile range (distance between the 25th and 75th percentile) of each biological marker, adjusted for age, sex and smoking status; [¶]: results were unchanged when restricting analyses to participants with current asthma.

adjusted for relevant potential confounders such as age, sex and smoking status, thought to be associated with the biomarkers we studied.

We did not find any significant association between erythrocyte SOD, GPX and catalase activities and asthma characteristics, in contrast with previous smaller studies (n∼30–150). In these studies, lower GPX level was consistently associated with asthma [13–15, 33]; however, for SOD and catalase, contrasting results with increased [13, 14, 33] or decreased [15, 34] levels according to asthma status were reported. Beside differences in technical measurements, discrepancies between results may be explained partly by differences in asthma outcomes (asthma, severe asthma or incident asthma) or in body compartments (erythrocytes, plasma or serum). The relationship between antioxidant enzymes and asthma is complex, involving several endogenous and exogenous factors that can influence biomarkers level. In addition, it is difficult to determine cross-sectionally whether variation in biomarker levels would be the cause or the consequence of asthma. Our null results, along with discrepant findings in the literature, suggest that biomarkers of response may not be the most relevant to study oxidative stress in asthma. However, longitudinal studies may provide more insight regarding this relationship.

We found a significant positive association between EBC 8-isoprostanes and ever asthma, consistent with previous studies including studies of children and adults reviewed in the article by Aldakheel *et al.* [12]. Overall, results suggest that 8-isoprostanes level may be a relevant biomarker for studying asthma. In addition, our findings confirm that EBC, which shows local production of free radicals [6], is an effective noninvasive method for measuring biomarkers related to oxidative stress.

In addition, we investigated the association between 8-isoprostanes and asthma outcomes among participants with ever asthma. Interestingly, among participants with asthma, we observed that those with childhood-onset asthma had higher 8-isoprostanes level than those with adult-onset asthma. However, we did not observe any significant association between 8-isoprostanes and other asthma outcomes. Furthermore, we confirm the lack of association between 8-isoprostanes and lung function or bronchial hyperresponsiveness, consistent with previous studies [35, 36]. We hypothesised that the longer a person has had asthma (for example, since childhood), the greater the tissue damage in the lungs, and that high 8-isoprostanes level reflects long-term damage, or the "background" of the disease, rather than its expression. However, we did not observe a significant association between asthma duration and 8-isoprostanes level (data not shown). Another hypothesis is that adult-onset asthma and childhood asthma, which are two different asthma phenotypes [4, 37], may have partly distinct biological mechanisms.

We found a negative association between FlOPs level and ever asthma, although this association was no longer significant after adjustment. Among participants with ever asthma, higher FlOPs level was significantly associated with poor asthma control, poor lung function and neutrophilic asthma. Consistently, we observed a significant positive association between FlOPs level, asthma attacks, use of any asthma treatments and use of ICS in the past 12 months. However, no significant association was found with current asthma, adult-onset asthma or respiratory symptoms. To the best of our knowledge, no study on the relationship between FlOPs and asthma has been published before. Nevertheless, a significant and positive association between FlOPs and others chronic inflammatory diseases such as chronic kidney disease (CKD) [17] or coronary heart disease [19] has been reported. Our results did not change after removing participants with history of CKD or cardiovascular diseases (data not shown).

Interestingly, a significant and positive association was observed between FlOPs and neutrophilic asthma, suggesting that FlOPs may be related to a nonallergic phenotype. Furthermore, in the literature, as in our analyses, FlOPs were shown to be associated with irritant exposures, such as tobacco smoke ([18] and online supplementary material) or occupational exposure to irritant chemicals (*e.g.* cleaning products) [29], which have also been associated with nonallergic asthma phenotypes [37–40].

Based on these findings, FlOPs appear to be a good biomarker for measuring oxidative stress, a pathophysiological mechanism related to several chronic diseases. In our study, some participants were not fasting at the time of blood collection and some potential confounders such as cholesterol were not taken into account. Although more studies are needed for the standardisation of FlOPs measurement, our findings suggest that this oxidation marker is linked to asthma expression.

Associations of 8-isoprostanes and FlOPs with asthma outcomes appeared to be discrepant in our study. Indeed, after adjustment, only 8-isoprostanes were associated with ever asthma and associations with asthma outcomes also differed for these two biomarkers among participants with ever asthma. The exact mechanisms explaining these different results are beyond the scope of our epidemiological study. As noted earlier, these biomarkers belong to different biological processes, and were measured in different compartments. 8-isoprostanes are the main biomarkers of the lipid peroxidation, measured in the exhaled breath condensate, probably reflecting the composition of the airway lining fluid [6], and may therefore better represent airway processes and reflect lung inflammation. FlOPs level is a biomarker of several oxidation processes, reflecting a mixture of oxidation products from DNA, proteins and lipids. This biomarker has been measured in blood, an easily available source of a large amount of antioxidant defences in the body [13], and can therefore better reflect oxidation at the systemic level.

In addition, none of the biomarkers of response to oxidative stress were correlated with biomarkers of damage, and no correlation was observed between 8-isoprostanes measured in EBC and FlOPs measured in blood (data not shown). Overall, all these results highlight the interest in studying biomarkers in several compartments belonging to different biological processes, and suggest that 8-isoprostanes and FlOPs to be complementary to the assessment of asthma.

Conclusion

In summary, our results suggest that EBC 8-isoprostanes seem to be involved in childhood-onset asthma and FlOPs seem to be linked to asthma expression and control in adults. Immediate clinical implications could not be inferred from this epidemiologic study. However, if replicated, our findings will suggest that FlOPs levels may help to identify asthmatics with higher asthma burden. Further research is needed, especially through longitudinal studies, to determine the potential interest of measuring FlOPs level in clinical practice.

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