ORIGINAL ARTICLE

# Inflammatory and Immune Effects of Pollution: Comparison Between Patients With Asthma and Healthy Individuals

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J Investig Allergol Clin Immunol 2026; Vol. 36(4)
doi: 10.18176/jiaci.1094

## Abstract

*Background:* The objective of this study was to assess inflammatory and immune responses in patients with asthma and healthy controls exposed to a polluted and a nonpolluted environment over a short period.
*Material and Methods:* We performed a randomized crossover study in patients with asthma (n=20) and in healthy controls (n=15). Participants were exposed for 2 hours to a polluted environment and, after 14 days, to a nonpolluted environment. Pollution levels were assessed at each exposure. Subsequently, serum levels of 8-isoprostane and glutathione peroxidase were measured as markers of oxidative stress, as were 48 cytokines involved in inflammation and the immune response.
*Results:* In the polluted environment, significantly higher levels of PM1, PM10, $NO_2$, NO, and CO were observed (*P*=.0026, .0337, <.0001, <.0001, and .0004, respectively) than in the nonpolluted environment. After exposure to a polluted environment, both groups (healthy controls and asthma patients) presented higher values of IL-17F (P=.0285 and .0348, respectively) and CSF2 (*P*=.0425 and 0.0305, respectively). After exposure to high levels of pollution, healthy controls presented reductions in glutathione peroxidase (with antioxidant activity), CSF3, HGF, and OSM (*P*=.0038, *P*=.0123, 0.0353, and 0.0256, respectively) and increased levels of IL-7, CXCL8, and CCL2 (*P*=.0015, 0.0119 and 0.0215, respectively). Asthma patients had higher serum levels of IL-1ß and IL-15 (*P*=.0232 and 0.0497, respectively).
*Conclusion:* Healthy individuals and asthma patients respond differently to exposure to pollutants. Healthy individuals are characterized by adaptive suppression of immune activity, whereas asthma patients present a more marked inflammatory response.

**Key words:** Pollution. Particulate matter. Immune profiling. Exposure. Real-time cohort.

## Resumen

*Antecedentes*: El objetivo de este estudio fue evaluar las respuestas inflamatorias e inmunológicas en pacientes asmáticos y controles sanos expuestos a un entorno contaminado o no contaminado durante un periodo de corta duración.
*Material y métodos:* Estudio cruzado aleatorizado en pacientes con asma (n=20) y controles sanos (n=15). Los participantes fueron expuestos durante 2 horas a un entorno contaminado y, tras 14 días, a un entorno no contaminado. En cada exposición se evaluaron los niveles de contaminantes. Posteriormente, se midieron los niveles séricos de 8-isoprostano y glutatión peroxidasa como marcadores de estrés oxidativo, así como 48 citocinas relacionadas con la respuesta inflamatoria e inmune.
*Resultados:* En el entorno contaminado se observaron niveles significativamente más altos de PM1, PM10, $NO_2$, NO y CO (p=0,0026; 0,0337; <0,0001; <0,0001 y 0,0004, respectivamente). Tras la exposición al entorno contaminado, ambos grupos presentaron valores más altos de IL17F (p=0,0285 y 0,0348) y CSF2 (p=0,0425 y 0,0305). Los controles sanos mostraron una reducción de glutatión peroxidasa (p=0,0038), CSF3, HGF y OSM (p=0,0123; 0,0353 y 0,0256, respectivamente), y un aumento de IL7, CXCL8 y CCL2 (p=0,0015; 0,0119 y 0,0215, respectivamente) tras la exposición al entorno contaminado. Los pacientes asmáticos presentaron niveles séricos más elevados de IL1b e IL15 (p=0,0232 y 0,0497, respectivamente).
*Conclusión:* En individuos sanos se observa una supresión adaptativa de la actividad inmunitaria ante la exposición a contaminantes, mientras que los asmáticos muestran una respuesta inflamatoria pronunciada.

**Palabras clave:** Contaminación. Material particulado. Perfil inmunológico. Exposición. Cohorte en tiempo real.

**Summary box**

- **What do we know about this topic?**
  Air pollution induces inflammation and oxidative stress, worsening asthma by triggering immune responses. While many studies confirm its harmful effects, the precise immunological pathways, especially the role of cytokines and oxidative balance, remain incompletely understood.
- **How does this study impact our current understanding and/or clinical management of this topic?**
  This study compares distinct short-term immune responses to pollution between asthma patients and healthy individuals, highlighting impaired antioxidant defense in asthma. Our findings support incorporating environmental risk assessments into asthma care and reinforce the need for targeted pollution reduction strategies.

## Introduction

Air pollution is one of the world's most pressing health problems. According to the World Health Organization, 90% of the world's population breathes polluted air [1]. Pollutants such as particulate matter (PM), nitrogen dioxide ($NO_2$), and sulfur dioxide ($SO_2$) can penetrate deep into the lungs, worsen pre-existing health conditions, and shorten overall life expectancy. In 2021, air pollution became the second leading risk factor for premature death, with 8.1 million associated deaths (including children) [2]. In fact, the elderly, children, and people with chronic conditions are especially vulnerable to air pollution.

Environmental pollution can have profound immunological effects on individuals with asthma and may exacerbate their condition. Pollutants produce inflammation and oxidative stress, have toxicological effects, and/or induce immunological changes [3-6]. They can also trigger immune responses that increase airway inflammation. In people with asthma, pollutants enhance the production of proinflammatory cytokines and activate immune cells such as mast cells and eosinophils, increasing sensitivity and worsening asthma symptoms [7]. Over time, repeated exposure to pollutants weakens the immune system's ability to regulate inflammation, making individuals more prone to frequent asthma attacks and infections [7].

Nevertheless, despite the significant amount of research carried out to date, much remains unknown about the inflammatory and immunological effects of environmental pollution on asthma. While it is known that pollution triggers inflammatory responses, the exact pathways through which pollutants synergize to worsen asthma remain unclear. Moreover, the role of oxidative stress in pollution-induced inflammation warrants more in-depth study. These gaps in our understanding highlight the need for further investigation into the complex relationship between environmental pollution and immune function in asthma.

The objective of this study was to compare inflammatory and immune responses in asthma patients and healthy controls exposed for a short time to a polluted and a nonpolluted environment.

## Materials and Methods

### *Study Population*

We performed a real-world randomized crossover study including nonsmoking patients aged ≥18 years diagnosed with asthma according to the Spanish Asthma Guidelines (GEMA) [8] and no other comorbidities who were referred to our center (Hospital Universitari Vall d'Hebrón, Barcelona, Spain) from June 2022 to July 2024 (n=20). All patients agreed to participate in the study. Patients had been diagnosed with perennial asthma, and bronchial hyperresponsiveness had been demonstrated by a methacholine test result <16 mg/mL. Patients with seasonal symptoms were excluded. Data from the clinical history and physical examination were analyzed. Patients were studied during periods of clinical stability, defined as the absence of exacerbations, respiratory infections, an Asthma Control Test (ACT) score >20, and no changes in treatment during the 4 weeks prior to the start of the study. During the study, participants maintained their usual asthma medication. A population of healthy volunteers (n=15) was included as a control group. To minimize the influence of diet and physical activity on oxidative stress parameters, participants were instructed to avoid intense physical activity and alcohol consumption. Participants were randomized to exposure sequences using simple randomization and assigned to the order of exposure (polluted or nonpolluted first) based on availability and operational feasibility at inclusion.

The study was approved by the local ethics committee (Hospital Vall d'Hebron Ethics Committee, approval [PR(AG)519/2022]), and all participants signed an informed consent document before entering the study.

### *Experimental Design*

Patients and volunteers were exposed for 2 hours (08:30-10:30) either to the environment in *La Ronda de Dalt*, an internal ring-road in Barcelona with a high level of diesel vehicle traffic (157 000 vehicles per day in 2021 [9]) and traffic jams during rush hour (09:00 and 17:00) and to the *Parque del Caracol*, a traffic-free area above Vall d'Hebrón Hospital. Based on previous findings [10], exposure sessions were separated by more than 2 weeks and were normally held on Thursdays. The sessions were held on dry days with an average temperature of 15 (5) ºC. Patients were driven to the Parque del Caracol in a diesel-powered van (journey time, 5 minutes). All sessions were held on weekdays between the months of October and March (2022 to 2024) to avoid pollen seasons.

### *Pollutant Assessment and Environmental Control*

A personal air quality monitor (Model AS520, Atmospheric Sensors Ltd; http://www.atmosphericsensors.

com [last accessed, 10/10/2024) was used to perform on-site environmental controls [11]. The monitor comprised electrochemical sensors measuring 20 mm in diameter to quantify carbon monoxide (CO), nitric oxide (NO), and $NO_2$ based on amperometry [12]. After quantification, calibration and a linear regression model (Eq. 1) were applied to convert raw sensor signals (mV) into mixing ratios (ppb). Finally, the data were corrected according to temperature, as follows:

$$[X]_{ref}=aWE_X+bAE_X+cWE_Y+d$$

where $[X]_{ref}$ is the reference measurement of pollutant X (ppb), a is the sensitivity of the working electrode ($ppbmV^{-1}$), $WE_X$ and $AE_X$ are the raw signal of the working and auxiliary electrodes, respectively (mV), b is the sensitivity of the auxiliary electrode ($ppbmV^{-1}$) (accounting for temperature), c is the cross-sensitivity with gas Y (ppbmV-1; *c=0* for CO and NO), WEY is the raw signal of the working electrode of the cross-sensitive gas *Y* (mV), and d is the intercept (ppb).

The monitor also integrates a commercial miniaturized optical particle counter (Alphasense OPC-N2; Alphasense Ltd, 2018), which enables real-time aerosol characterization and classification into PM1, PM2.5, and PM10 [13]. Finally, data were corrected according to relative humidity [13,14].

### *Functional Respiratory Parameters*

All participants underwent spirometry testing after each exposure period. Pulmonary function was assessed using a Viasys pulmonary function system (VYaire-Vyntus Body), and the Z-score was calculated in compliance with the guidelines and theoretical values established by the European Respiratory Society and the American Thoracic Society [15,16].

### *Biomarker Determination*

Blood samples were collected after each exposure period in 8.5-mL (16×100 mm) SST™ II Advance Plus Blood Collection Tubes (BD Vacutainer) and then fractionated into 500-µL aliquots in SafeSeal 1.5-mL reaction tubes (SARSTEDT). The samples were frozen at −80°C until analysis.

Targeted proteomic profiling was performed in serum samples using Olink (Olink Bioscience AB). A total of 48 serum proteins were measured, including $T_H1$, $T_H2$, and $T_H17$ proteins and inflammation-related proteins (CCL38, IL-33, CXCL12, OLR1, IL-27, IL-2, CXCL9, TGFA, IL-1B, IL-6, IL-4, TNFSF12, TSLP, CCL11, HGF, FLT3LG, IL-17F, IL-7, IL-13, IL-18, CCL13, TNFSF10, CXCL10, IFNγ, IL-10, CCL19, TNF, IL-15, CCL3, CXCL8, MMP12, CSF2, CSF3, VEGFA, IL-17C, EGF, CCL2, IL-17A, OSM, CSF1, CCL4, CXCL11, LTA, CCL7, and MMP1). Blood samples were assayed in a 96-well plate containing 50 µL of sample using the Target 48 Cytokine Panel, which is based on proximity extension assays and characterized by its high sensitivity and reproducibility and low cross-reactivity. Data processing and quality control have been described elsewhere [17].

### *Statistical Analysis*

The normality of the data distribution was evaluated using the Shapiro–Wilk test. For categorical variables, data were analyzed using the $\chi^2$ or Fisher exact test and are expressed as absolute numbers and their corresponding percentages. For quantitative variables, data were analyzed using the Mann-Whitney test and are shown as medians and ranges. The analyses were conducted using GraphPad Prism 10 for Windows (version 10.01, GraphPad Software Inc) Differences with a *P* value<.05 (2-tailed) were considered significant.

## Results

### *Pollutants*

Pollutant concentrations were determined by repeated personal air quality monitor measurements after each minute of exposure following the parameters described above. Median (range) daily concentrations (µg/m³) for PM1, PM2.5, PM10, $NO_2$, NO, and CO in the polluted area were 3.454 (1.264-10.02), 4.744 (1.516-16.77), 11.55 (1.682-26.24), 60.64 (25.46-183.3), 8.034 (nondetectable [ND]-32.29), and 364.9 (246.8-2036), respectively. Median (range) daily concentrations (µg/m³) for PM1, PM2.5, PM10, $NO_2$, NO, and CO in the nonpolluted area were 3.414 (1.213-4.982), 5.672 (1.399-18.12), 8.563 (1.382-61.45), 12.75 (ND-70.71), 0.861 (ND-2.436), and 209.4 (74.13-673.7), respectively.

The values were higher at the polluted site than at the nonpolluted site, with significant differences reported for

**Table 1.** Level of Pollutants in the 2 Areas Studied.

| | Polluted area | Nonpolluted area | *P* Value |
|---|---|---|---|
| PM1, µg/m³ | | | .0026 |
| Median | 3.454 | 3.414 | |
| Range | 1.264-10.02 | 1.213-4.982 | |
| PM2.5, µg/m³ | | | .6155 |
| Median | 4.744 | 5.672 | |
| Range | 1.516-16.77 | 1.399-18.12 | |
| PM10, µg/m³ | | | .0337 |
| Median | 11.55 | 8.563 | |
| Range | 1.682-26.24 | 1.382-61.45 | |
| Nitrogen dioxide, µg/m³ | | | <.0001 |
| Median | 60.64 | 12.75 | |
| Range | 25.46-183.3 | ND-70.71 | |
| Nitrogen monoxide, µg/m³ | | | <.0001 |
| Median | 8.034 | 0.861 | |
| Range | ND-32.29 | ND-2.436 | |
| Carbon monoxide, µg/m³ | | | .0004 |
| Median | 364.9 | 209.4 | |
| Range | 246.8-2036 | 74.13-673.7 | |

Abbreviations: ND, nondetectable (considered as 0 for statistical purposes).

PM1, PM10, $NO_2$, NO, and CO (*P*=.0026, *P*=.0337, *P*<.0001, *P*<.0001, and *P*=.0004, respectively) (Table 1).

### *Study Population*

The study population comprised 15 healthy controls with a median age of 27 (25-69) years and 20 asthma patients with a median age of 55 (29-73) years (*P*=.0002). Women accounted for 60% of the controls and 55% of the asthma patients.

Eleven patients (55%) had severe asthma, 8 (40%) moderate asthma, and 1 (5%) mild asthma. The median follow-up of these patients was 6 (2-13) years. Eleven asthma patients (55%) presented atopy. Medication is shown in Table 2.

### *Functional Respiratory Parameters*

As expected, asthma patients presented lower values for $FEV_1$ (both % predicted and z-score), FVC (both % predicted

**Table 2.** Demographic Variables and Lung Function Parameters.

| Variable | Healthy controls (n=15) | | | Asthma patients (n=20) | | *P* Value |
|---|---|---|---|---|---|---|
| Median (range) age, y | 27 (25-69) | | | 55 (29-73) | | .0002 |
| Female sex, No. (%) | 9 (60) | | | 11 (55) | | .7674 |
| Asthma severity, No. (%) | | | | | | |
| Severe | NA | | | 11 (55) | | |
| Moderate | | | | 8 (40) | | |
| Mild | | | | 1 (5) | | |
| Median (range) follow-up, y | NA | | | 6 (2-13) | | |
| Atopy, No. (%) | NA | | | 11 (55) | | |
| Medication | | | | | | |
| SABA on demand | NA | | | 15 (75) | | |
| LABA on demand | | | | 9 (45) | | |
| SAMA on demand | | | | 4 (20) | | |
| Inhaled corticosteroids | | | | 19 (95) | | |
| LABA | | | | 20 (100) | | |
| LAMA | | | | 12 (60) | | |
| Oral corticosteroids | | | | 2 (10) | | |
| Montelukast | | | | 9 (45) | | |
| Azithromycin | | | | 2 (10) | | |
| Biological treatment | | | | 6 (30) | | |
| **Pulmonary function test** | **Polluted** | **Nonpolluted** | ***P* Value** | **Polluted** | **Nonpolluted** | ***P* Value** |
| $FEV_1$, median (IQR) | | | | | | |
| % predicted | 95.5 (–86.8 to 103.3) | 93.2 (91.2-100.5) | .7984 | 83.35 (73.03-98.25) | 80.90 (70.83-95.63) | .8675 |
| z-score | 0.1 (–0.32 to 0.31) | –0.08 (–0.25 to 0.46) | .9674 | –0.7 (–1.35 to 0.37) | –0.9 (–1.68 to 0.22) | .9171 |
| FVC, median (IQR) | | | | | | |
| % predicted | 96.40 (81.60-103.5) | 97.10 (85-104.4) | .6592 | 92.80 (77.65-101.2) | 88.80 (72.93-98.43) | .9572 |
| z-score | 0.28 (–0.78 to 0.65) | 0.29 (–0.58 to 0.90) | .6984 | –0.15 (–0.98 to 0.42) | –0.39 (–1.26 to 0.44) | .7553 |
| $FEV_1$/FVC, median (IQR) | | | | | | |
| % predicted | 99.4 (91-104.9) | 99.30 (89.60-105.9) | .7009 | 91.40 (84.05-99.88) | 89.25 (85.60-97.20) | .9372 |
| z-score | –0.03 (–1.24 to 0.71) | –0.16 (–1.41 to 1.01) | .9616 | –0.99 (–1.76 to 0.1) | –1.483 (–1.68 to 0.47) | .4430 |

Abbreviations: LABA, long-acting ß-agonist; LAMA, long-acting muscarinic antagonists; NA, not applicable; SABA, short-acting ß-agonist; SAMA, short-acting muscarinic antagonist.

and z-score), and $FEV_1$/FVC (both % predicted and z-score). Pulmonary function tests showed no significant differences between the polluted and nonpolluted sites in either group (Table 2).

### *Biomarkers of Oxidative Stress*

Figure 1 shows glutathione peroxidase (GPX) activity (A) and the concentration of 8-isoprostane (pg/mL) (B) in serum samples. The control group presented lower GPX activity after exposure to the polluted site than the nonpolluted site (*P*=.0038). Although no significant differences in 8-isoprostane concentration were observed between exposures in either group, asthma patients presented higher levels of this marker after exposure to the polluted environment than healthy controls.

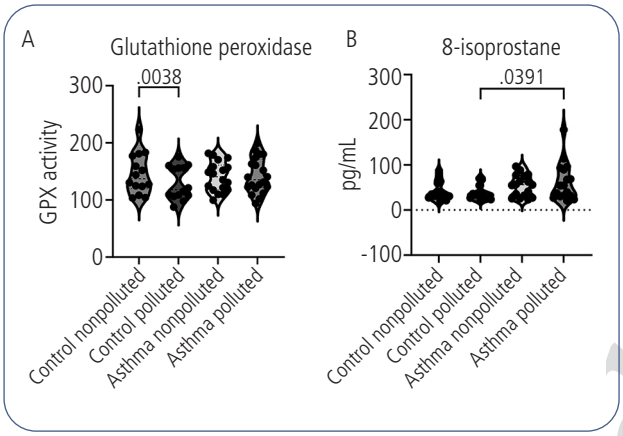


**Figure 1.** Biomarkers of oxidative stress. The groups comprised a control group of healthy volunteers and a group of patients with mild/moderate asthma after exposure to a nonpolluted site (nonpolluted) and after exposure to a polluted site (polluted). Individual and median values of glutathione peroxidase (GPX) activity (A) and 8-isoprostane concentration (pg/mL) are shown (B).

### *Cytokines*

A volcano plot was used as an exploratory tool to identify which cytokines were differentially expressed depending on exposure to the pollutant. Figure 2 compares the change in expression by log2 fold change (log2FC) and the statistical significance of these differences by log10 (*P* value) for the control group polluted vs nonpolluted (A) and for the asthma group polluted vs nonpolluted (B). The log2FC values ranged between −1 and 1, indicating moderate changes in expression, with positive log2FC values indicating upregulation and negative values indicating downregulation. Cytokines surpassing the significance threshold (*P*<.05) were considered to be differentially expressed. After exposure to the polluted site, the control group presented upregulated IL-7, CXCL8, CCL2, IL-17F, and CSF2 (*P*=.0015, *P*=.0119, *P*=.0215, *P*=.0285, and *P*=.0425, respectively) and downregulated CSF3, OSM, and HGF (*P*=.0123, *P*=.0256, and *P*=.0353, respectively), while the asthma group presented upregulated IL-1ß , CSF2, IL-17F, and IL-15 (*P*=.0232, *P*=.0305, *P*=.0348, and *P*=.0497, respectively) in comparison to the nonpolluted site.

Figure 3 shows the median and individual serum levels of IL-1β (A), IL-17F (B), IL-7 (C), and IL-15 (D). After exposure to the polluted site, IL-1β and IL-15 levels were higher in patients with asthma (*P*=.0232 and .0497, respectively), the control group had higher concentrations of IL-7 (*P*=.0015), and both groups had higher concentrations of IL-17F (*P*=.0285 for the control group and *P*=.0348 for the asthma group) than those exposed to the nonpolluted site.

After exposure to the polluted site, the control group presented increased concentrations of CXCL8 and CCL2

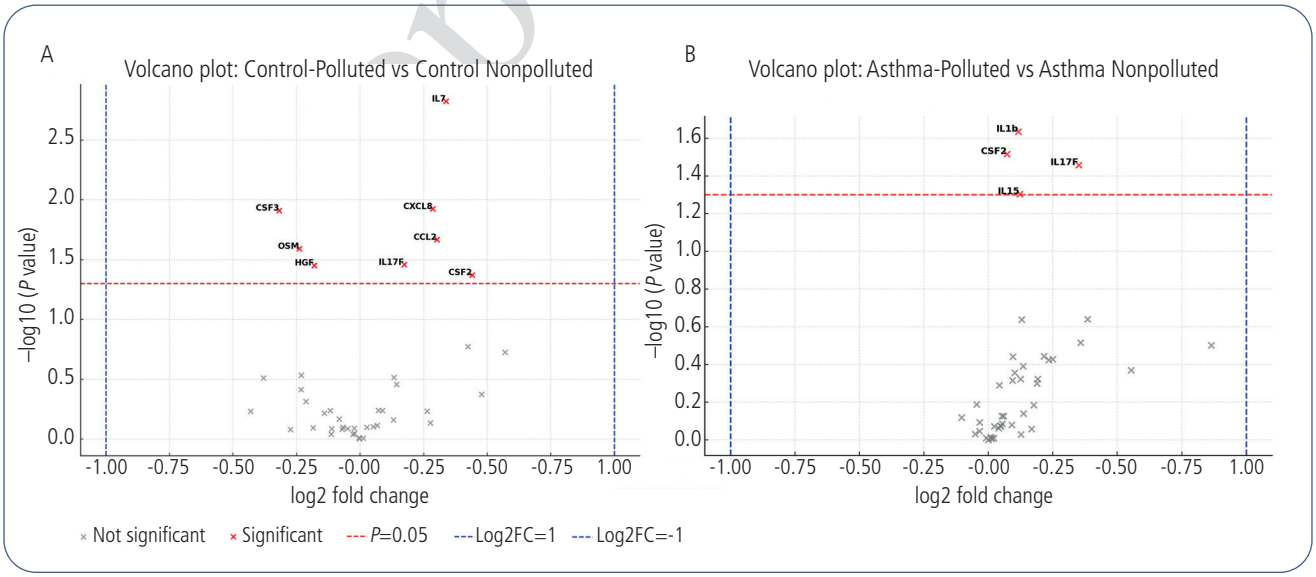


**Figure 2.** Volcano plot illustrating the differential expression of cytokines. The x-axis represents the log2 fold change (log2FC), while the y-axis represents the –log10 (*P* value). Significant changes in cytokine expression (*P*<.05) are marked in red; positive log2FC values indicate upregulation and negative values indicate downregulation. Cytokine concentrations (pg/mL) were compared between control group nonpolluted vs polluted (A) and asthma nonpolluted vs polluted (B).



doi: 10.18176/jiaci.1094

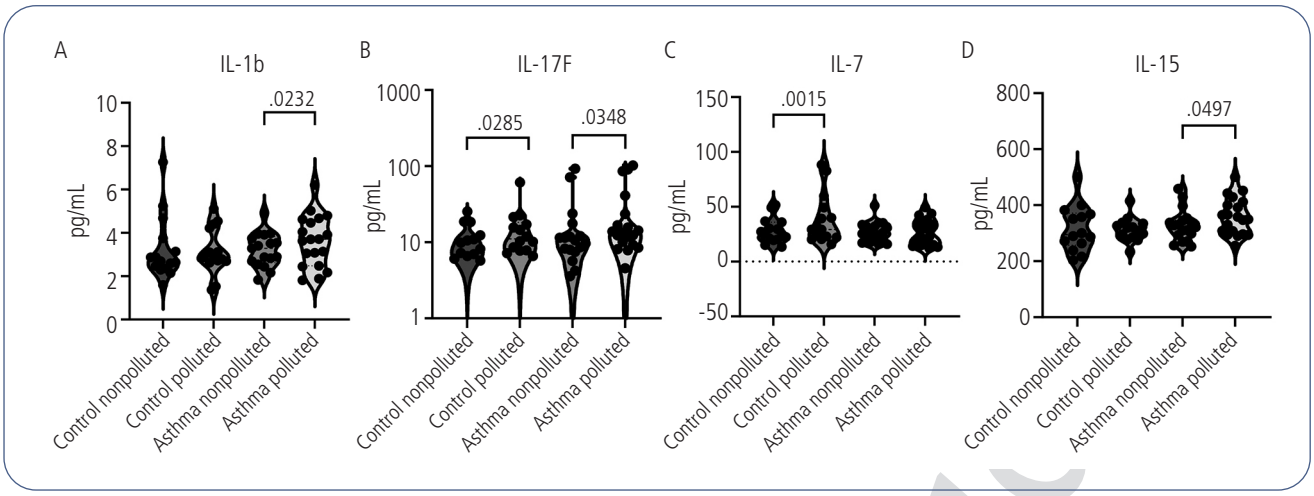


**Figure 3.** Proinflammatory cytokines. The experimental groups shown are the same as in Figure 1. Median and individual concentrations (pg/mL) of IL-1ß (A), IL-17F (B), IL-7 (C), and IL-15 (D).

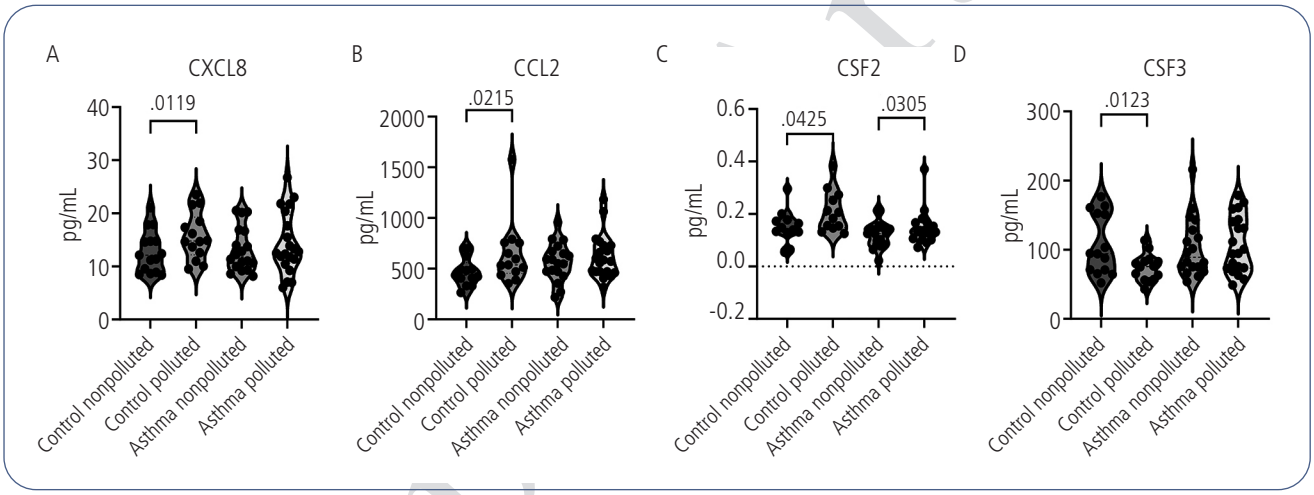


**Figure 4.** Chemoattractant cytokines. The experimental groups shown are the same as in Figure 1. Median and individual concentrations (pg/mL) of CXCL8 (A), CCL2 (B), CSF2 (C), and CSF3 (D).

(*P*=.0119 and *P*=.0215, respectively), and both groups presented increased concentrations of CSF2 (*P*=.0425 and *P*=.0305, respectively) than the groups at the nonpolluted site. However, the control group presented a lower concentration of CSF3 after exposure to the polluted site than to the nonpolluted site (*P*=.0123) (Figure 4).

The control group presented higher HGF and OSM concentrations after exposure to the nonpolluted site than after exposure to the polluted site (*P*=.0353 and 0.0256 respectively) (Figure 5).

## Discussion

The present study examines the inflammatory effects of real-life exposure to traffic-related air pollution in healthy people and in asthma patients. The study demonstrates that short-term exposure to a polluted environment produces different responses in the 2 populations. Asthma patients presented an inflammatory response with increases in proinflammatory cytokines such as IL-1ß , IL-15, and IL-17F. In healthy individuals, the immune response was triggered with increases in chemokines capable of activating the recruitment of monocytes (CCL2) and macrophages (CSF2) or to actively maintain a stable population of naive T cells (IL-7) as a defense mechanism against the inhalation of pollutants.

Exposure to traffic-related air pollution can have immediate and significant effects on the lung, even in healthy individuals. Traffic pollution can lead to increased airway inflammation and oxidative stress and reduce lung capacity [18]. The fine particles in traffic emissions can penetrate deep into the lungs, causing irritation and triggering an immune response that releases proinflammatory cytokines and chemokines. Even short-term exposure to traffic pollution has been associated with impaired lung function, increased airway hyperresponsiveness, and enhanced susceptibility to respiratory infections [19].

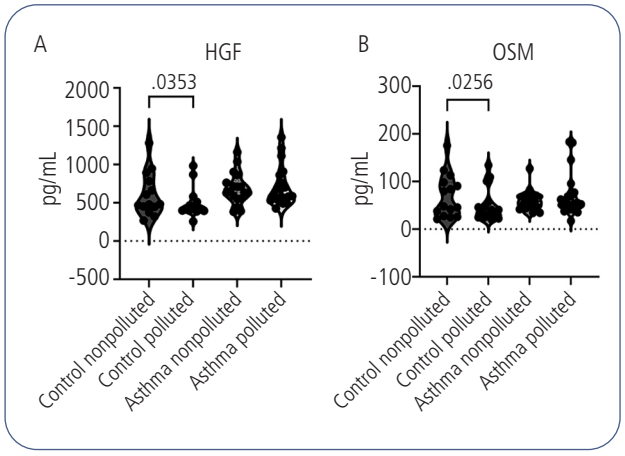


**Figure 5.** Tissue remodeling cytokines. The experimental groups shown are the same as in Figure 1. Median and individual concentrations (pg/mL) of HGF (A) and OSM (B).

In the present study, GPX levels decreased in healthy individuals after a short exposure to a traffic-related polluted environment. GPX is a key enzyme in the body's defense against oxidative stress, and its role becomes particularly significant in individuals exposed to environmental pollutants [20]. Exposure to air pollutants increases the production of reactive oxygen species (ROS), which can exacerbate inflammatory responses and oxidative damage in the lungs [18]. GPX activity is particularly important in preventing inflammation and tissue damage, supporting the body's ability to recover from the harmful effects of environmental toxins [21]. GPX activity may fall upon exposure to environmental pollutants owing to the increased oxidative stress and inflammation that pollutants induce, thus explaining the reduction observed in healthy individuals in the present study. Nevertheless, we observed no changes in the levels of GPX in asthma patients, who already had lower baseline levels of GPX in both environments than healthy controls. In this regard, the activity of GPX tends to be reduced in persons with asthma, impairing the body's ability to neutralize ROS and increasing its vulnerability to further damage from pollutants. Reduced GPX activity in asthma has been associated with more pronounced airway inflammation, greater airway hyperresponsiveness, and more frequent asthma exacerbations [22,23]. For their part, levels of 8-isoprostane, a marker of oxidative stress, appeared to be higher in individuals with asthma, although the difference did not reach statistical significance. Our results seem to indicate that the antioxidant defense system of asthma patients is compromised, probably owing to chronic inflammation and oxidative stress within the airways, whereas healthy individuals can regulate oxidative stress more effectively. This supposition is corroborated by the fact that asthma patients have a higher level of 8-isoprotane, a marker of oxidative stress, when exposed to a polluted environment, while healthy controls maintain lower levels of this marker.

After short-term exposure to traffic-related pollutants, asthma patients presented increased levels of proinflammatory cytokines such as IL-1β, IL-15, and IL-17F. IL-1β plays a crucial role in the inflammatory responses associated with environmental pollution and can exacerbate asthma symptoms [24]. Moreover, elevated levels of IL-1β have been linked to the activation of immune cells that contribute to chronic airway inflammation [25]. For its part, IL-15 has emerged as an important cytokine in the context of environmental pollution and its impact on asthma. Recent studies indicate that exposure to airborne pollutants can lead to significantly increased IL-15 levels, which may also contribute to the exacerbation of asthma symptoms through the activation of immune cells, particularly natural killer cells and T cells [26]. Elevated IL-15 concentrations enhance the survival and proliferation of these cells, leading to heightened inflammation in the airways [26]. Additionally, IL-15 has been linked to the development of airway hyperresponsiveness [27]. IL-17F is a proinflammatory cytokine, and exposure to air pollutants can upregulate its expression in the lungs [28]. This increase in IL-17F levels is linked to the activation of $T_H17$ cells, which are responsible for mediating inflammatory responses in the airways. In individuals with asthma, elevated IL-17F levels in response to pollutants may contribute to more severe inflammation and airway hyperresponsiveness [29]. In the present study, this cytokine is also elevated in healthy individuals. In this regard, while IL-17F is typically associated with inflammatory diseases such as asthma, its levels can also rise in healthy individuals exposed to pollutants, contributing to increased oxidative stress and airway inflammation. This elevation in IL-17F may act as an early immune response to environmental stressors, suggesting a role in the development of airway hyperresponsiveness and other respiratory conditions over time. An increase in IL-7 was also observed in healthy individuals but not in asthma patients, who presented low levels of this cytokine in both scenarios. IL-7 plays a crucial role in the development, survival, and homeostasis of T cells, particularly in maintaining a stable population of naive T cells. The lower levels of IL-7 in asthma patients may result from an imbalance in a cytokine environment where elevated levels of other proinflammatory cytokines predominate, potentially suppressing IL-7 expression.

After short exposure to traffic-related pollutants, healthy individuals presented increased levels of chemokines, such as CXCL8 and CCL2. CXCL8, also known as IL-8, plays a key role in the recruitment and activation of neutrophils during inflammatory responses. Several studies have shown that exposure to pollutants increases CXCL8 levels in the respiratory system, leading to enhanced neutrophil migration and the release of proinflammatory mediators. CCL2, also known as monocyte chemoattractant protein-1, is a chemokine that plays a significant role in the recruitment of monocytes and macrophages to sites of inflammation. This upregulation of CCL2 can activate the immune system, resulting in chronic low-grade inflammation in the lungs. While healthy individuals may not develop immediate symptoms, chronic exposure to pollutants, and the subsequent elevation of CXCL8 and CCL2 could contribute to airway inflammation, oxidative stress, and potential long-term damage to lung tissue, thus increasing susceptibility to respiratory diseases such as asthma.

In both groups of individuals, an increase in levels of CSF2 was observed after short exposure to pollutants. CSF2 is a cytokine involved in the regulation of hematopoiesis and immune cell function, particularly in the differentiation and

activation of granulocytes and macrophages. Upregulation of CSF2 enhances the production and activation of immune cells, particularly macrophages and neutrophils, contributing to inflammation and oxidative stress in the respiratory system. Increased levels of CSF2 in response to pollutants have also been associated with airway remodeling and the persistence of inflammation, a finding that underlines its role in the early immune response to environmental stressors.

After short-term exposure to pollutants, healthy individuals presented reductions in hepatocyte growth factor (HGF), osmotin, and CSF3. However, no variations in these biomarkers were observed in individuals with asthma. The differential response between healthy individuals and asthma patients suggests distinct mechanisms of immune regulation and inflammation. In fact, HGF is a key regulator of tissue repair and regeneration and is known for its ability to promote cell proliferation, migration, and wound healing. It may actually play a dual role, supporting tissue repair while also potentially contributing to the excessive inflammation and fibrosis that develop with chronic exposure [30,31]. Osmotin is a cytokine with proinflammatory and immunomodulatory properties, and its expression is influenced by environmental stressors. It is primarily involved in mediating inflammation and modulating immune cell activity, particularly by promoting the recruitment of neutrophils and enhancing the production of other proinflammatory cytokines. In the lungs, osmotin can contribute to airway inflammation, oxidative stress, and tissue remodeling, processes commonly associated with chronic respiratory conditions. Moreover, CSF3 is an important survival and proliferation factor for neutrophils and macrophages. In healthy individuals, reductions in these cytokines may reflect adaptive suppression of immune activity to mitigate the excessive inflammation triggered by pollutants. Conversely, in asthma patients, pre-existing chronic inflammation and heightened immune activation may maintain baseline or elevated levels of these cytokines. This resilience in cytokine levels among asthma patients could indicate a dysregulated inflammatory set-point, at which the immune system is less responsive to external suppressive signals.

The absence of differences in pulmonary function despite significant immune alterations may be due to the fact that short-term exposure to pollution triggers immune responses before measurable lung function changes occur. Pulmonary function parameters are relatively stable and may undergo significant alterations without prolonged or repeated exposures. Additionally, individual variability and adaptive mechanisms could play a role.

Our study captures the acute inflammatory effects of short-term (2-hour) exposure to polluted environments, revealing immediate immune alterations. Prolonged exposure to pollutants may lead to persistent oxidative stress and systemic inflammation, which have been linked to the progression of respiratory diseases, accelerated lung function decline, and increased asthma risk. Additionally, chronic exposure can alter immune homeostasis, potentially increasing susceptibility to infections and exacerbating conditions such as asthma. More evidence is needed to understand how these responses differ between individuals with asthma and healthy individuals over prolonged exposure periods, as well as the long-term effects that may arise in both groups.

Our study is subject to a series of limitations. First, the population is restricted to the inhabitants of a large city; it would have been interesting to compare the results obtained with a rural population not continuously exposed to pollution. Second, although our study design, which includes randomization and a crossover approach, helps minimize selection bias and intraindividual variability, the markers of oxidative stress and cytokines measured are not specific to the effect of the air pollutants, and the impact of other causative factors or confounders cannot be ruled out. Unmeasured environmental and personal variables, such as diet, stress, and other lifestyle factors, could contribute to oxidative stress and cytokine variability and modulate immune responses. Although participants received general instructions to minimize these factors, residual confounding cannot be ruled out. Third, the significant difference in age between controls and patients may have affected the outcomes of inflammation. In addition, the results may have been influenced by differences in asthma severity. However, since this is a pre-post study in which participants are compared individually, these limitations could be mitigated. Fourth, variations in the values for markers of inflammation may have been influenced by sex differences in immune responses. However, the absence of sex-specific analyses is a potential limitation. Future research should explore these differences to better understand their impact on inflammation. Fifth, while the real-world exposure settings enhance the ecological validity of our findings, we acknowledge that travel to the nonpolluted area by diesel-powered transportation could introduce residual exposure to pollution. Although we selected areas with lower traffic density, some exposure may have occurred during transit. Notwithstanding, the real-world setting still provides valuable insights into the impact of urban pollution. Finally, the sample size was small; future studies should corroborate these results in larger populations.

In conclusion, our study demonstrates that healthy individuals and asthma patients respond differently to a polluted environment, suggesting distinct mechanisms of immune regulation and inflammation in both populations. Healthy individuals are characterized by adaptive suppression of immune activity to mitigate the excessive inflammation triggered by pollutants. Conversely, asthma patients present a more marked inflammatory response, probably owing to pre-existing chronic inflammation.

Our findings contribute to the growing body of evidence associating exposure to air pollution with immune dysregulation, particularly in individuals with asthma. From a public health perspective, these results highlight the need for targeted strategies to minimize exposure to pollution in vulnerable populations. Potential interventions include the implementation of air quality monitoring systems, the promotion of clean indoor environments through air filtration and ventilation strategies, and public policies aimed at reducing traffic-related emissions. In clinical practice, health care providers should consider integrating environmental risk assessments into asthma management plans, advising patients on exposure reduction measures such as avoiding outdoor activities during peak pollution hours or using protective masks when necessary. Future research should explore the effectiveness of these strategies in mitigating the inflammatory effects of air pollution in individuals with asthma.

*Funding*

DE is a researcher supported by the Rio Hortega program from Instituto de Salud Carlos III (CM23/00174). FP is a researcher supported by the Contratos Predoctorales de Formación en Investigación en Salud programme (PFIS) from Instituto de Salud Carlos III (FI22/00262). This project received funding from the Fundació Catalana de Pneumologia (FUCAP), Instituto de Salud Carlos III (PI21/01346), Fondo Europeo de Desarrollo Regional (FEDER), and Sociedad Española de Patología Respiratoria (SEPAR). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

*Conflicts of Interest*

XM has received fees as a speaker, scientific advisor, and participant in clinical studies from AstraZeneca, Boehringer Ingelheim, Chiesi, Faes, Gebro, GlaxoSmithKline, Menarini, Mundifarma, Novartis, Sanofi, and Teva.

In the last 3 years, DE has received fees as a speaker from AstraZeneca, Bial, and Chiesi. He has received financial aid from Sanofi, Bial, Gebro, Menarini, and Chiesi for attending congresses.

In the last 3 years, IO declares having received honoraria for participating as a speaker in meetings sponsored by AstraZeneca, Boehringer-Ingelheim, Chiesi, and Novartis and as a consultant for AstraZeneca, GlaxoSmithKline, Puretech, and Sanofi. He has received financial aid from AstraZeneca, Bial, and Chiesi for attending congresses and grants from Sanofi for research projects.

In the last 3 years, CR has received speaker fees from AstraZeneca and Chiesi. He has received financial aid from Sanofi, Bial, Gebro, Menarini, and Chiesi for attending congresses.

In the last 3 years, FP has received speaker fees from Chiesi. She has received financial aid from Sanofi and Boehringer Ingelheim for attending congresses.

The remaining authors declare that they have no conflicts of interest.

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*Manuscript received January 26, 2025; accepted for publication April 15, 2025.*

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