Air pollution and biomarkers of systemic inflammation and tissue repair in COPD patients

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ABSTRACT The origin(s) of systemic inflammation in patients with chronic obstructive pulmonary disease (COPD) is unclear. We investigated the impact of exposure to ambient air pollution on systemic biomarkers of inflammation (C-reactive protein (CRP), tumour necrosis factor-α, interleukin (IL)-6, IL-8 and fibrinogen) and tissue repair (hepatocyte growth factor (HGF)) in 242 clinically stable COPD patients (mean age 67.8 years and forced expiratory volume in 1 s 71.3% predicted) in Barcelona, Spain, in 2004–2006.

A spatiotemporal exposure assessment framework was applied to predict ambient nitrogen dioxide (NO_2) and levels of particles with a 50% cut-off aerodynamic diameter of 2.5 μm ($PM_{2.5}$) at each participant's home address during 10 periods of 24 h (lags 1–10) and 1 year prior to the blood sampling date. We used linear regression models to estimate associations between biomarkers and exposure levels.

An interquartile range (IQR) increase in NO_2 exposure in lag 5 was associated with 51%, 10% and 9% increases in CRP, fibrinogen and HGF levels respectively. We also observed 12% and 8% increases in IL-8 associated with an IQR increase in NO_2 exposure in lag 3 and over the year before sampling, respectively. These increases were larger in former smokers. The results for $PM_{2.5}$ were not conclusive.

These results show that exposure to ambient NO_2 increases systemic inflammation in COPD patients, especially in former smokers.

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NO_2 exposure increases levels of systemic inflammation biomarkers in COPD patients, especially in former smokers http://ow.ly/sRKnX

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Introduction

Chronic obstructive pulmonary disease (COPD) is often associated with systemic inflammation identified by increased circulatory levels of inflammatory biomarkers, such as C-reactive protein (CRP), tumour necrosis factor (TNF)-α, interleukin (IL)-6, IL-8 and fibrinogen [1]. This systemic inflammation is clinically relevant, since recent research has shown that COPD patients with persistent systemic inflammation have more comorbidities, particularly cardiovascular diseases (CVD), suffer more frequent exacerbations and have worse prognosis [1–6].

The origin(s) of systemic inflammation in COPD is unclear. In healthy volunteers and in patients with CVD, exposure to air pollution is associated with an increase in the circulatory levels of inflammatory biomarkers, including TNF-α, IL6, IL8 and CRP [2, 7–14]. To our knowledge, the available evidence on such an association in COPD patients is very scarce [15]. Circumstantial evidence, however, indicates that short-term exposure to air pollution can trigger an exacerbation of COPD, and that long-term exposure to air pollution can contribute to the development and progression of COPD [2, 7, 16, 17].

Hepatocyte growth factor (HGF) is a pulmotrophic factor that participates in lung repair [18, 19], and its circulatory level increases in COPD [18, 19]. Although the tissue damage due to exposure to air pollution could have a potential impact on the production of HGF, we are not aware of any available experimental or epidemiological study analysing such an impact either in COPD patients or in non-COPD individuals.

We hypothesised that exposure to air pollution enhances systemic inflammation and repair processes in COPD. To test this hypothesis, we investigated the relationship of exposure to gaseous (nitrogen dioxide (NO_2) and particulate (particles with a 50% cut-off aerodynamic diameter of 2.5 μm ($PM_{2.5}$)) air pollution on circulatory biomarkers of inflammation (CRP, TNF-α, IL-6, IL-8 and fibrinogen) and tissue repair (HGF) in clinically stable COPD patients.

Methods

Overview

We investigated among COPD patients whether subjects with higher air pollution exposure had higher levels of systemic inflammation. A single blood sample was available for each subject. We evaluated both long-term and short-term exposure to air pollution. Long-term exposure was assessed as the annual average and short-term exposure by the 24-h average prior to the blood sampling date. To evaluate potential delayed effect, short-term effects were studied for up to 10 days before blood sampling. We also explored whether such effects are different between current and former smokers and between obese and non-obese participants.

Study design, participants and ethics

According to our study question on the systemic inflammatory response to air pollution in COPD patients (and not comparing such response between subjects with and without COPD), this cross-sectional analysis only included cases of COPD, in line with similar previous studies that relied merely on cases of COPD [15] or CVD [11–14]. Our analysis was therefore based on data from 251 COPD patients obtained by the Phenotype and Course of COPD (PAC-COPD) Study, whose design and methodology have been described in detail elsewhere [20, 21]. In brief, it recruited COPD patients during their first hospital admission because of an exacerbation of the disease in seven tertiary university hospitals across Barcelona metropolitan area (Spain) between January 2004 and March 2006. All epidemiological, clinical, functional and biological data were collected when patients had been clinically stable for $\geqslant$3 months after hospital discharge. The diagnosis of COPD was established according to the American Thoracic Society/European Respiratory Society definition of post-bronchodilator forced expiratory volume in 1 s (FEV_1) to forced vital capacity ratio $\leqslant$0.70 [22]. Forced spirometry was performed following international guidelines with the reference

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values of a Mediterranean population [23]. Patients aged <45 years and those with cancer, residual extensive tuberculosis lesions of more than one third of the pulmonary parenchyma, pneumonectomy and/or pneumoconiosis were excluded. The research protocol was approved by the Ethics Committees of all the participating hospitals, and written informed consent was obtained from all participants.

Blood samples

Our study relied on a single blood sample obtained from each participant. The blood sample was available for 242 (96%) out of the 251 recruited participants who were included in our analyses. Participants without blood sample (n=9) were not different (Fisher's exact or Mann–Whitney U-test p-values >0.05) from those with blood sample in terms of age, sex, body mass index (BMI), FEV_1, education level, smoking status and pollutant exposure levels. A venous blood sample (20 mL) was obtained by peripheral venipuncture in the early morning hours after fasting overnight. Current smokers were asked to refrain from smoking for $\geqslant$8 h before sampling. 30 min after withdrawal, blood was centrifuged at 2000–3000 rpm (720–1620 × *g*) for 10 min. Serum was then separated and stored in cryotubes at -80°C until analysis. All serum analyses (except fibrinogen) were carried out centrally at Hospital Universitari Son Dureta (Palma de Mallorca, Spain). Serum levels of CRP were determined by high-sensitivity immunonephelometry (Dade Behring, Marburg, Germany) and those of HGF were measured using a commercially available ELISA kit (Amersham Pharmacia, Biotech UK Ltd, Little Chalfont, UK). The serum concentrations of IL-6, IL-8 and TNF-α were determined by high-sensitivity ELISA (Biosource, Camarillo, CA, USA). The lower limit of quantification (LLQ) values of these assays were 0.16 $mg \cdot L^{-1}$, 0.104 $pg \cdot mL^{-1}$, 0.10 $pg \cdot mL^{-1}$, 0.09 $pg \cdot mL^{-1}$ and 40 $pg \cdot mL^{-1}$ for CRP, IL-6, IL-8, TNF-α and HGF, respectively. To those participants with a biomarker level below the LLQ, we assigned a nominal level of half of the LLQ value for that biomarker, in order to avoid a downward bias of the data [4]. All assays were performed in duplicate (intra-assay variation <10%) and reported values correspond to the average of these two determinations. Serum fibrinogen levels were determined in each hospital using the Clauss method as part of routine blood exams [24].

Assessment of exposure to air pollution

The home addresses of the study participants were geocoded to the exact address according to postal code, street name and house number. To assess the exposure of each participant to air pollution, we first estimated the ambient levels of NO_2 and $PM_{2.5}$ at the home address by means of land use regression (LUR) models developed as part of the European Study of Cohorts for Air Pollution Effects (ESCAPE) [25, 26]. We then added a temporal component to these spatial estimates, enabling us to predict ambient levels of NO_2 and $PM_{2.5}$ at each geocoded home address (*i.e.* spatial component) for each day of the study period (*i.e.* temporal component). Further details on this spatiotemporal exposure assessment are described in the online supplementary material (exposure assessment methodology and supplementary table S1) and have been published elsewhere [25, 26].

For each participant, we estimated ambient NO_2 and $PM_{2.5}$ levels separately at the home address during 10 windows of exposure, using pollutant levels for 10 periods of 24 h (lags 1–10) prior to the single blood sampling date (*i.e.* lag 1 is the first day before the sampling, lag 2 is the second day before the sampling, *etc.*).

Statistical analyses

Main effect

We used linear regression models to estimate the effects of NO_2 and $PM_{2.5}$ exposures (separately) on the log-transformed circulating level of each biomarker in each lag (one at a time). Analyses were adjusted for age, sex, education level (none or primary/secondary/university), BMI and recruiting hospital. To facilitate the comparison of associations between two pollutants and 10 exposure windows (lags), results are reported as percentage change in the mean concentration of each biomarker associated with one interquartile range (IQR) increase in NO_2 or $PM_{2.5}$ exposure levels in each lag.

Effect of smoking and obesity

To investigate whether any observed association between air pollution and biomarker levels differs between current and former smokers and between obese (BMI $\geqslant$30 $kg \cdot m^{-2}$) and non-obese participants, 1) we used the likelihood ratio test to compare models with and without interaction term between air pollution and smoking as well as air pollution and obesity (one at a time); and 2) we stratified the main effect analyses based on smoking status and obesity. For these analyses, we used NO_2 exposure levels averaged over lags 3–7, the period for which we found the strongest associations for the evaluated biomarkers. The stratified analyses according to the smoking status were adjusted for age, sex, education level, BMI and recruiting hospital, while those based on obesity were adjusted for age, sex, education level, smoking status and recruiting hospital.

Effect of long-term air pollution exposure

Our main effect analyses were based on short-term exposure (lags 1–10) to air pollution. To explore the impact of long-term air pollution exposure on the biomarkers, we repeated the aforementioned main effect analysis using mean NO_2 and $PM_{2.5}$ exposure levels averaged over the year prior to the sampling date [27].

Sensitivity analysis

Out of 242 available blood samples, 108 (45.4%) had TNF-α levels below the LLQ. For the rest of the biomarkers studied, the percentage of samples with levels below the LLQ ranged between 0% and 8%. To further explore the impact of the samples with levels below the LLQ (to which we assigned half of the LLQ value), we dichotomised TNF-α levels by the median and used logistic regression models with an identical set of exposure variables and covariates as above but with this binary variable as outcome.

Results

Table 1 describes characteristics of study participants and levels of biomarkers. The Spearman's correlation coefficient (rho) between NO_2 exposure levels in different lags ranged from 0.35 (between lag 10 and lag 1) to 0.79 (between lag 7 and lag 6). For $PM_{2.5}$, correlations were weaker than those observed for NO_2, with rho values ranging between 0.02 and 0.43. As presented in table S2, correlations between CRP, fibrinogen and IL-6 were modest (rho 0.27–0.56), whereas the remaining biomarkers were not correlated or weakly correlated (with the exception of CRP and HGF, where rho was 0.30).

Effect of air pollution on inflammatory markers

As presented in figure 1 (and table S3), we observed a clear pattern of associations between NO_2 exposure and levels of CRP, fibrinogen and HGF: the associations between lag 1 to lag 10 peaked at lag 5, with a statistically significant increase for CRP in lags 4–7, fibrinogen in lags 3–8 and HGF in lags 4–8. An IQR increase in NO_2 exposure in lag 5 (*i.e.* the fifth day before blood sampling) was associated with 51% (95% CI 22–88%), 10% (95% CI 5–15%) and 9% (95% CI 3–6%) increase in serum levels of CRP, fibrinogen and HGF, respectively. TNF-α showed a similar pattern, with a peak at lag 4, although the associations did not reach statistical significance. For IL-6 and IL-8 we did not observe a clear pattern across the lags, but we found up to 12% (95% CI 1–24%) increase in IL-8 associated with an IQR increase in exposure to lag 3.

For $PM_{2.5}$, we did not observe a clear pattern of associations across the lags (table S4) and the associations did not attain statistical significance, with the exception of the 52% (95% CI 16–99%) and 37% (95% CI 6–77%) increase in TNF-α associated with an IQR increase in $PM_{2.5}$ exposure in lags 1 and 7, respectively.

These analyses were further adjusted for the following factors, one at a time: the severity of airflow limitation, according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) spirometric classification [29]; smoking duration (years); cumulative smoking exposure (pack-years); passive smoking ($h \cdot week^{-1}$); use of inhaled corticosteroids (yes/no); use of statins (yes/no); alcohol intake ($g \cdot day^{-1}$); lifetime history of occupational exposure to dust, chemicals and smoke, based on job codes obtained from face-to-face interview and linked to a job exposure matrix providing semi-quantitative estimation of exposure (low/medium/high); physical activity, according to the summary index of the Yale Physical Activity Survey [28]; presence of cardiovascular comorbidities (yes/no); living inside *versus* outside the city of Barcelona; having a window at home opening to a street (yes/no); marital status (couple/noncouple); ambient temperature (at the same lag as air pollution); and season of blood sampling (summer/winter). This did not change our findings significantly, except for the adjustment of ambient temperature, which strengthened the associations of TNF-α with NO_2 exposure in lag 4 (40% (95% CI 3–89%)) and lag 5 (33% (95% CI 1–74%)).

Effect of smoking and obesity

In our study sample, 152 (62.8%) patients were former smokers and 90 (37.2%) were current smokers. After stratification of analyses for NO_2 exposure (averaged over lags 3–7) according to smoking status, we generally observed larger increases in biomarkers in former compared to current smokers (fig. 2 and table S5); however, the likelihood ratio test of interaction between smoking status and NO_2 exposure was statistically significant only for CRP ($p<0.01$). An IQR increase in NO_2 during lags 3–7 was associated with -17% (95% CI -47–28%) change in CRP levels in current smokers and a 111% (95% CI 56–184%) increase in former smokers.

Of our study participants, 84 (34.7%) were obese and 158 (65.3%) were not obese. In stratified analyses based on obesity, the patterns of associations between air pollution and biomarkers for obese and non-obese participants were different among the biomarkers (fig. 3 and table S6), with the likelihood ratio test of interaction being statistically significant only for IL-8 ($p=0.02$).

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TABLE 1 Characteristics of participants

Variable	Description
Study participants n	242
Age years	67.8±8.6
Sex	
Female	13 (5.4)
Male	229 (94.6)
Education level	
None or primary school	109 (45.0)
Secondary school	102 (42.2)
University	31 (12.8)
Marital status	
Couple	195 (80.6)
Noncouple	47 (19.4)
Body mass index kg·m^{-2}	28.1±4.6
Post-bronchodilator FEV_1 % predicted	71.3±16.7
GOLD class	
I (mild)	10 (4.1)
II (moderate)	111 (45.9)
III (severe)	98 (40.5)
IV (very severe)	23 (9.5)
Use of corticosteroid inhaler	
Yes	94 (38.8)
No	148 (61.2)
Use of statins	
Yes	40 (16.5)
No	202 (83.5)
Season of blood sampling	
Autumn/winter	90 (37.2)
Spring/summer	152 (62.8)
Occupational exposure history	
Low	78 (37.3)
Medium	71 (34.0)
High	60 (28.7)
Smoking status	
Former smoker	152 (62.8)
Current smoker	90 (37.2)
Smoking duration years	44.8±12.5
Smoking intensity pack-years	0.4±0.7
Passive smoking h·week^{-1}	2.6±4.7
Alcohol intake g·day^{-1}	16.0±20.3
Physical activity[#]	39.9±21.0
Cardiovascular comorbidities	
Myocardial infarction	30 (12.4)
Congestive heart failure	15 (6.2)
Peripheral vascular disease	27 (11.2)
Cerebrovascular disease	12 (5.0)
CRP mg·L^{-1}	8.9±18.5
TNF-α pg·mL^{-1}	0.9±1.4
IL-6 pg·mL^{-1}	1.4±1.3
IL-8 pg·mL^{-1}	5.3±3.8
Fibrinogen g·L^{-1}	4.2±1.2
HGF pg·mL^{-1}	1041.0±491.6

Data are presented as mean±SD or n (%), unless otherwise stated. FEV_1: forced expiratory volume in 1 s; GOLD: Global Initiative for Chronic Obstructive Lung Disease; CRP: C-reactive protein; TNF: tumour necrosis factor; IL: interleukin; HGF: hepatocyte growth factor. [#]: according to the Yale Physical Activity Survey [28].

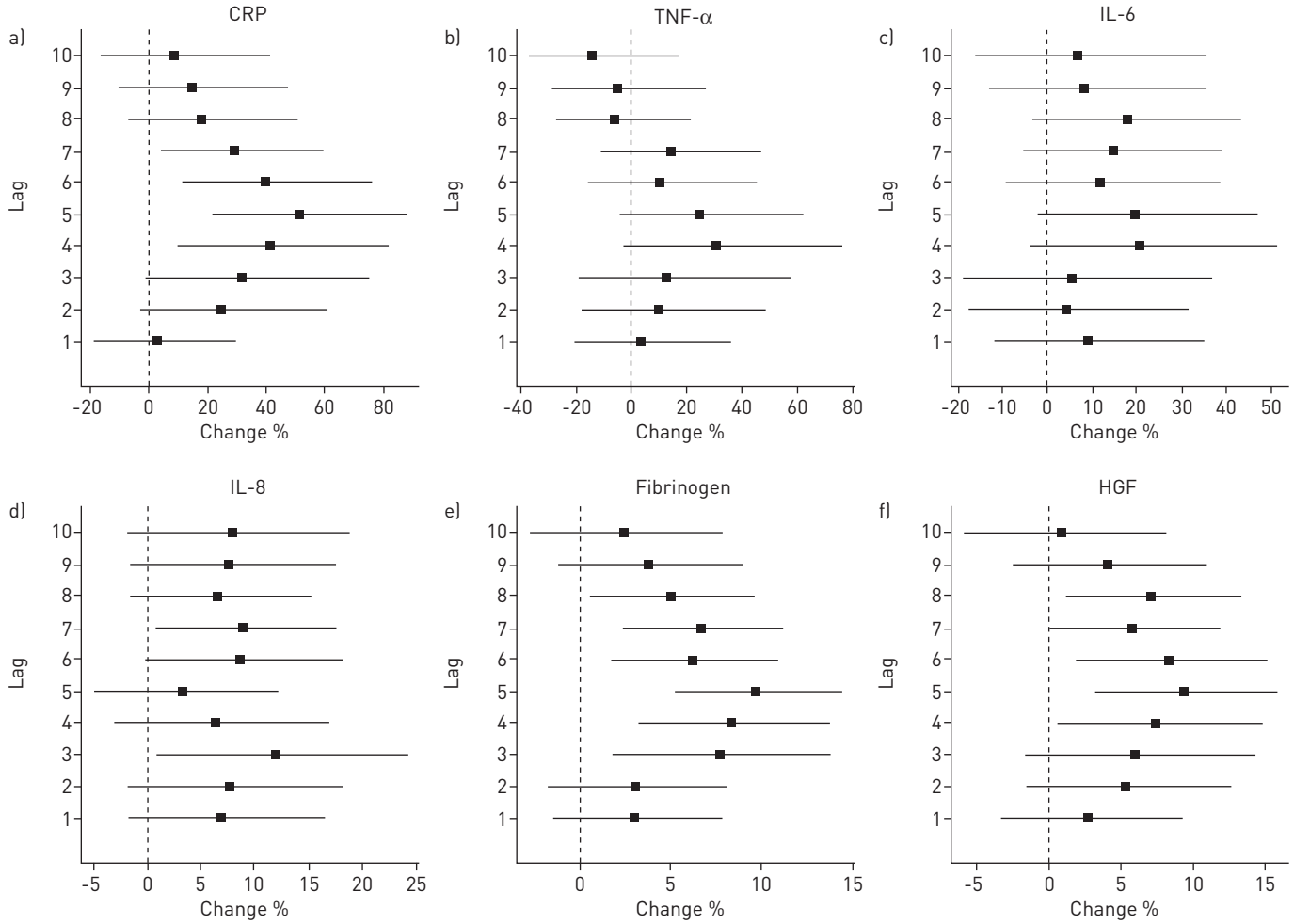

FIGURE 1 Percentage change (95% CI) in biomarkers of inflammation and tissue repair in clinically stable chronic obstructive pulmonary disease patients. a) C-reactive protein (CRP), b) tumour necrosis factor (TNF)-α, c) interleukin (IL)-6, d) IL-8, e) fibrinogen and f) hepatocyte growth factor (HGF). Data are adjusted for age, sex, body mass index, education level and recruiting hospital. Changes associated with one interquartile range increase in nitrogen dioxide exposure are shown separately for each lag.

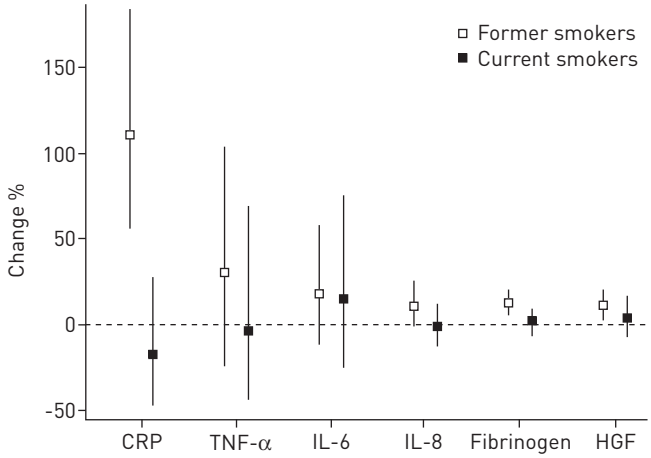

FIGURE 2 Percentage change (95% CI) in biomarkers of inflammation and tissue repair in clinically stable chronic obstructive pulmonary disease patients, according to smoking status. Data are adjusted for age, sex, body mass index, education level and recruiting hospital. Changes associated with one interquartile range increase in nitrogen dioxide exposure during lags 3–7 are shown separately for current and former smokers. CRP: C-reactive protein; TNF: tumour necrosis factor; IL: interleukin; HGF: hepatocyte growth factor.

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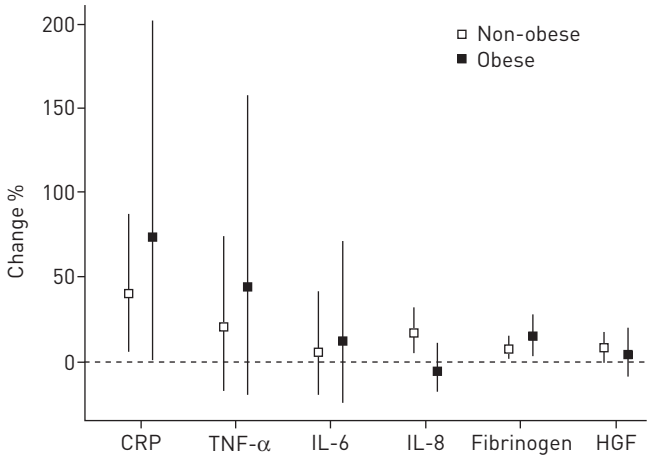

FIGURE 3 Percentage change (95% CI) in biomarkers of inflammation and tissue repair in clinically stable chronic obstructive pulmonary disease patients, according to obesity. Data are adjusted for age, sex, smoking status, education level and recruiting hospital. Changes associated with one interquartile range increase in nitrogen dioxide exposure during lags 3–7 are shown separately for obese and non-obese participants. CRP: C-reactive protein; TNF: tumour necrosis factor; IL: interleukin; HGF: hepatocyte growth factor.

We further tested the interaction of air pollution and severity of airflow limitation (GOLD classes), cardiovascular comorbidities and statin use (one at a time), but did not observe any significant interaction. We did not explore the effect of smoking status and obesity on the associations between biomarkers and $PM_{2.5}$ exposure because the main effect findings for this exposure were not conclusive.

Long-term effects of air pollution on inflammatory markers

The median (IQR) values of the mean NO_2 and $PM_{2.5}$ exposure levels during the year prior to blood sampling were 43.1 (14.5) $\mu g \cdot m^{-3}$ and 15.8 (3.0) $\mu g \cdot m^{-3}$, respectively. An IQR increase in 1-year exposure to NO_2 was associated with an 8% (95% CI 1–16%) increase in IL-8 (table 2). For the rest of the biomarkers studied, as well as for $PM_{2.5}$ exposure, results were not conclusive (table 2).

Sensitivity analysis

For NO_2 exposure, the direction and pattern of associations using dichotomised TNF-α as outcome were generally comparable with those of the main analyses (table S7), suggesting robustness of our findings to the inclusion of samples with TNF-α levels below LLQ. In contrast, the associations between $PM_{2.5}$ exposure in lags 1 and 7 and TNF-α (that were statistically significant in the main effect analyses) lost their statistical significance.

Discussion

To our knowledge, this study is one of the first to investigate the impact of exposure to ambient air pollution on biomarkers of systemic inflammation and tissue repair in COPD. We used baseline

TABLE 2 Change in biomarkers associated with long-term air pollution exposure levels

Biomarker	NO_2	$PM_{2.5}$
CRP	-1.6 (-18.9–19.5)	-4.6 (-20.0–13.6)
TNF-α	7.8 (-14.2–35.5)	0.0 (-18.6–23.0)
IL-6	6.6 (-10.6–27)	9.5 (-6.5–28.3)
IL-8	7.8 (0.5–15.6)*	3.2 (-3.2–10.0)
Fibrinogen	0.9 (-2.8–4.8)	-0.6 (-4.0–2.9)
HGF	0.2 (-4.8–5.4)	1.4 (-3.2–6.2)

Data are presented as percentage change (95% CI). Changes shown are associated with one interquartile range increase in mean exposure levels of nitrogen dioxide (NO_2) and particles with a 50% cut-off aerodynamic diameter of 2.5 μm ($PM_{2.5}$) during the year prior to the sampling date. Data are adjusted for age, sex, body mass index, education level and recruiting hospital. CRP: C-reactive protein; TNF: tumour necrosis factor; IL: interleukin; HGF: hepatocyte growth factor. *: $p<0.05$.

demographic and outcome data from an established cohort of COPD patients, together with a validated spatiotemporal exposure assessment method capable of predicting ambient NO_2 levels at the home address of each participant for each day of the study period. We showed that short-term (lags 3–7) exposure to NO_2, but not $PM_{2.5}$, is associated with significant increases in serum levels of CRP, IL-8, fibrinogen and HGF in patients with clinically stable COPD, particularly in former smokers. These associations were diluted when long-term (1-year) exposure was considered.

Previous studies

HILDEBRANDT *et al.* [15] studied the impact of NO_2, nitric oxide (NO) and particulate air pollution on biomarkers of coagulation and inflammation during lags 0–4 days in a sample of 38 males with chronic pulmonary disease (including 34 cases of COPD). Their biomarkers included CRP and fibrinogen but not IL-6, IL-8, TNF-α or HGF. They found increased levels of fibrinogen in association with exposure to particulate air pollutants and NO in lags of 3–4 days before blood sampling, which was consistent with our observed peak of fibrinogen, CRP and IL-8 levels associated with NO_2 exposure at lags of 4–5 days. These findings are also in line with findings of a previous study in healthy subjects and patients with asthma, chronic bronchitis and COPD, showing that the highest probability of requiring a doctor visit because of respiratory symptoms was associated with exposure to NO_2 in lag 4 [17]. That study also reported that the highest probability of respiratory-related doctor visit in male COPD patients was associated with NO_2 exposures during lags 2–4, which is akin to our findings, considering that 95% of our study sample were male [17].

Our observed associations between biomarkers of inflammation and NO_2 exposure are consistent with findings of *in vitro* studies showing an increase in synthesis of TNF-α, IL-6 and IL-8 by human bronchial epithelial cells exposed to NO_2 [30, 31]. TNF-α and IL-6 are acute phase proteins known to upregulate the synthesis of CRP, fibrinogen and other pro-inflammatory/pro-thrombotic mediators [2]. Furthermore, the associations we found are consistent with previous epidemiological studies reporting increased levels of CRP, IL-8 and fibrinogen after exposure to ambient NO_2 in healthy individuals [27, 32–34] and in patients with CVD [11–14].

We also observed that short-term NO_2 exposure is associated with increased circulatory levels of HGF. We are not aware of any toxicological or epidemiological study on the impact of air pollution on HGF levels in individuals with or without COPD and our finding therefore requires further confirmation by such studies.

The analysis of potential long-term (1-year) effects was inconclusive, except for IL-8 levels. The only study available so far on the effect of long-term NO_2 exposure on inflammatory markers showed increased IL-6 levels associated with 5- and 30-year exposures in patients with myocardial infarction [27].

Although the interaction between smoking status and NO_2 exposure was statistically significant only for CRP, our stratified analyses showed that the increase in biomarker levels associated with NO_2 exposure were between 1.5- to 11.5-fold larger in former than in current smokers. This observation is in agreement with previous studies reporting larger lung function decrement and more respiratory symptoms in response to ozone challenge in nonsmokers compared to smokers [35, 36], which has been suggested to be explained by upregulation of antioxidant mediators in smokers [37, 38]. Consistently, it has been reported that inflammatory response to air pollution in asthmatic children is more evident in those not exposed to environmental tobacco smoke (ETS) compared to those exposed to ETS [38]. Air pollution and ETS have been suggested to act as competing risk factors and saturation of common biological pathways of the effects of air pollution and ETS in those exposed to ETS has been proposed as a potential explanation for these findings [39]. However, the available evidence on the interaction between air pollution and smoking in COPD shows discrepancies. While a number of studies have not supported such an interaction [40–42], others have reported synergism of air pollution and smoking in COPD [43, 44].

Previous studies suggest a link between adipose tissue dysfunction, systemic inflammation and COPD [45, 46]. We assessed a potential interaction between obesity and air pollution in relation to biomarkers of systemic inflammation, with mixed results. While for CRP, TNF-α, IL-6 and fibrinogen we observed a larger increase in obese compared with in non-obese participants, for IL-8 and HGF we observed the opposite, with a statistically significant interaction only for IL-8. These findings require further confirmation by future studies.

The available body of evidence is suggestive of a sex difference in pathogenesis and prognosis of COPD [4, 46, 47]. Because of the small number of female participants in our study ($n=13$), it was not feasible to carry out sex-stratified analyses to explore potential sex differences in our associations. The levels of biomarkers were not different between our female and male participants (Mann–Whitney U-test, $p>0.05$);

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however, females had higher exposure levels (NO_2 exposure during lags 3–7) compared to the male participants (Mann–Whitney U-test, p=0.02).

For $PM_{2.5}$ exposure, we found increased levels of TNF-α in relation to exposures in lags 1 and 7. However, this must be interpreted cautiously because about half the blood samples had TNF-α levels below the LLQ and the sensitivity analysis using dichotomised TNF-α did not support these associations.

Clinical implications

Recent research has shown that systemic inflammation in patients with COPD is associated with increased mortality, exacerbation rate and comorbid conditions like CVD, skeletal muscle dysfunction, osteoporosis and diabetes [1–6]. The origin of systemic inflammation in COPD is unclear. Our study showed that, in these patients, exposure to NO_2 is associated with short-term increased serum levels of CRP, IL-8 and fibrinogen. A recent study reported that current smoking, older age, increased BMI, poor quality of life and the severity of airflow limitation present were all significantly and independently associated with the presence of systemic inflammation in COPD [4]. Our results extend these findings and show that environmental exposure is another potential factor to consider in this context. In addition, our observed increased circulatory levels of HGF could potentially reflect an attempt to repair lung damage induced by NO_2. This finding may suggest a trend towards an enhanced remodelling of lungs in COPD patients due to exposure to air pollution that, in the long term, could possibly contribute to the structural abnormalities observed in the airways of patients with COPD.

Potential limitations

First, by design, our cross-sectional study had a limited capability for causal inferences and was prone to selection bias. Secondly, clinical data was obtained in the period 2004-2006 whereas LUR models estimating the exposure were constructed using 2009 data. Thus, the temporal adjustment of LUR spatial estimates of pollutant levels assumed that the city spatial surface and the spatial distribution of pollutants remained constant over the study period. This is not unlikely, since studies in Italy, the UK, the Netherlands and Canada have documented stability of the spatial contrast and validity of LUR estimates over long periods [48–51]. Finally, we used ambient pollutant levels at the home addresses of participants as a surrogate for personal exposure. This approach overlooks participants' time–activity patterns, which might have resulted in some degree of exposure misclassification. For example, time spent in traffic/commuting could potentially have a considerable impact on personal exposure to air pollution for which we did not have data.

Conclusions

Short-term exposure to NO_2 was associated with increased levels of biomarkers of systemic inflammation (CRP, IL-8 and fibrinogen) and tissue repair (HGF), particularly in former smokers. We observed mixed findings for the interaction of this exposure and obesity. Short-term $PM_{2.5}$ exposure and long-term (1-year) exposure to NO_2 and $PM_{2.5}$ had less conclusive effects. These findings could provide new insights into the mechanisms of systemic inflammation in COPD [52]. Considering the increased mortality, exacerbation rate and comorbidities associated with systemic inflammation in COPD patients, our findings can offer clues for targeted interventions aimed at improving prognosis in these patients.

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