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Air pollution exposure, SARS-CoV-2 infection, and immune response in a cohort of healthcare workers of a large university hospital in Milan, Italy

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ABSTRACT

Several studies have examined the possible relationship between air pollutants and the risk of COVID-19 but most returned controversial findings. We tried to assess the association between (short- and long-term) exposure to particulate and gaseous pollutants, SARS-CoV-2 infections, and immune response in a population of healthcare workers (HCWs) with well-characterized individual data. We collected occupational and clinical characteristics of all HCWs who performed a nasopharyngeal swab (NPS) for detecting SARS-CoV-2 at the Policlinico Hospital in Milan (Lombardy, Italy) between February 24, 2020 (day after first documented case of COVID-19 in our hospital) and December 26, 2020 (day before start of the vaccination campaign). Each subject was assigned daily average levels of particulate matter ≤10 μm (PM10), nitrogen dioxide (NO_2), and ozone (O_3) retrieved from the air quality monitoring station closest to his/her residential address. Air pollution data were treated as time-dependent variables, generating person-days at risk. Multivariate Poisson regression models were fit to evaluate the rate of positive NPS and to assess the association between air pollution and antibody titer among NPS-positive HCWs. Among 3712 included HCWs, 635 (17.1%) had at least one positive NPS. A 10 $\mu g/m^3$ increase in NO_2 average concentration in the four days preceding NPS was associated with a higher risk of testing positive [Incidence Rate Ratio (IRR) = 1.08, 95% confidence interval (CI): 1.01; 1.16)]. When considering a 1 $\mu g/m^3$ increase in 2019 annual NO_2 average, we observed a higher risk of infection (IRR: 1.02, 95%CI: 1.00; 1.03) and an increased antibody titer (+2.4%, 95%CI: 1.1; 3.6%). Findings on PM10 and O_3 were less consistent and, differently from NO_2, were not confirmed in multipollutant models. Our study increases the body of evidence suggesting an active role of air pollution exposure on SARS-CoV-2 infection and confirms the importance of implementing pollution reduction policies to improve public health.

1. Introduction

Lombardy (Northern Italy, 10 million people) was the region most affected by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection in Italy, with more than 4 million cases as of May 2023. Focusing on 2020 only (i.e., before the start of the vaccination campaign), the total number of cases in Lombardy was above 470.000, with more than 25.000 deaths, representing 22% and 33% of total national counts, respectively (https://epiprev.it/apps/made.php). Several factors have been evoked as potential contributors to SARS-CoV-2 spread and COVID-19 diffusion, including climatic, environmental, demographic, socioeconomic, and geographical factors (Brągoszewska and Mainka, 2022; Burbank, 2023; Coccia, 2020, 2021a, 2021b; Cortes-Ramirez et al., 2022). In Lombardy, its well-known high levels of air pollution have been suggested as a possible cause of the high incidence of SARS-CoV-2 infections experienced in this region.

Air pollution could be able to influence the pathogenesis of SARS-CoV-2 infection through different factors. Exposure to particulate

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matter (PM) could have a function on the spread of the virus by acting as a transport vector in the environment and by facilitating its entry into the respiratory tract (Martelletti and Martelletti, 2020). Evidence derived from both epidemiological and laboratory studies suggest that air pollution can enhance susceptibility to respiratory infections through different biological mechanisms which include alteration of the protective barriers of the respiratory tract, modification of the host defense functions of macrophages, dysregulation in the airway microbiome, modification of the cellular receptors used by virus to cause infections (Monoson et al., 2023). Interestingly, some data have highlighted the ability of PM to increase the expression of ACE2, the receptor that allows the virus to enter the cell, and of TMPRSS2, the transmembrane serine protease that allows the virus to bind to the receptor (Woodby et al., 2021). Furthermore, there are numerous works in the literature that document the role of air pollution on the immune system. The main air pollutants can determine a weakening of the immune response through the production of free radicals and interfere with the modulation of the inflammatory response (Re and Facchini, 2020). The involved mechanisms are still not completely unveiled: on the one hand air pollution can enhance the innate immune and inflammatory responses (Monoson et al., 2023), on the other hand it can alter the antiviral factor activation (Marín-Palma et al., 2023) and affect the adaptive immune response impairing T-cell polarization (activation) (Tuazon et al., 2022). Finally, both air pollution and the virus share the lung as the main target organ and the presence of both elements could amplify their harmful effects on the lung.

Numerous studies have examined the possible relationship between environmental pollutants and the risk of COVID-19. The most recent evidence has been summarized in a meta-analysis (Zang et al., 2022) and systematic reviews including a considerable number of articles (Hernandez Carballo et al., 2022; Marquès and Domingo, 2022). The meta-analysis examined 35 studies of which only 3 were cohort studies based on individual data, 17 were ecological studies and 15 were time-series studies.

The incidence of COVID-19 was positively associated with increases of NO_2, PM2.5 and SO_2 for both short and long-term exposures. Mortality from COVID-19 was positively associated with long-term exposure to NO_2 and PM2.5. However, a subgroup analysis highlighted that the positive associations for long-term exposure to NO_2 and incidence or mortality were limited only to low- or middle-income regions, suggesting the possible confounding role of factors such as access to care and health system capacity to deal with the pandemic.

In general, epidemiological studies that have attempted to estimate the relationship between air pollution and COVID-19 incidence/mortality suffer from important limitations.

Most are ecological studies that used aggregated data for both exposure and outcome assessment across an extremely broad geographic domain, thus making inference at the individual level difficult. These studies are also easily affected by residual confounding, since main confounders (race, sex, age, smoking, etc.) are also assessed at group level and not at individual level, and lack control of other factors such as population density, frequency and availability of tests (testing rate), and stage of the pandemic. For example, in the first months of the pandemic, since the availability of tests was much lower than the number of infected subjects, diagnostic accuracy was not high.

Furthermore, testing was more likely in urban areas with greater health resources. At the same time, air pollution levels are higher in urban areas, and this may lead to an overestimation of the association between infection rate and air pollution as highlighted by some studies which have shown associations between the probability of being tested and PM2.5 levels (Chadeau-Hyam et al., 2020).

In addition, most studies examining incidence have relied on data derived from surveillance systems. Such data are inherently biased by cases seeking treatment and obtaining testing, thus introducing selection bias when other factors leading to an association between air pollution and testing rates (as mentioned previously) are not accounted for in the analysis. Even in the most recent study conducted on the entire Italian territory, data on diagnosed SARS-CoV-2 infections were retrieved from surveillance systems (Stafoggia et al., 2023).

Finally, pollution exposure estimates may be subject to measurement errors due to misclassification of a variety of factors (e.g., incomplete land coverage of air quality monitoring networks, failure to adequately capture the individual heterogeneity of exposure), which add further uncertainty to the obtained estimates.

To adequately investigate the association between exposure to air pollution and the incidence of SARS-CoV-2 infection, well-designed longitudinal studies with individual-level data are therefore needed, to minimize the effect of the aforementioned biases (Villeneuve and Goldberg, 2020). A few longitudinal studies with individual level-data, which we will discuss later, have been recently published but returned inconsistent findings (Kogevinas et al., 2021; Nobile et al., 2022; Sheridan et al., 2022; Veronesi et al., 2022).

In this context, we tried to assess the nature of the association between (both short- and long-term) exposure to particulate and gaseous pollutants, SARS-CoV-2 infections, and response of the immune system in a well-characterized study population with availability of individual data and exposure measurements.

2. Materials and methods

2.1. Study population

The study population consists of healthcare workers (HCWs) of the Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico of Milan, Italy, who performed at least one nasopharyngeal swab (NPS) for SARS-CoV-2 infection between February 24, 2020 (the day after the first documented case of COVID-19 occurred in our hospital) and December 26, 2020 (the day before the start of the vaccination campaign). Reasons to perform NPS were previous contact with a COVID-19 case, presence of symptoms, or periodic systematic testing within a mandatory surveillance-screening program for all HCWs. At the time of each NPS, all HCWs had to fill-in a questionnaire to collect sociodemographic data, information on lifestyle habits, residence and medical history.

2.2. Outcome definition and study design

We focused on two different outcomes:

- SARS-CoV-2 infection (either symptomatic or not) defined as the first positive NPS;
- Immune response evaluated through antibody titer (i.e., the level of IgG antibodies against SARS-CoV-2) from a serological assay offered freely to all hospital HCWs.

To investigate the association between short-term air pollution exposure and probability of SARS-CoV-2 infection, we treated our population as a cohort, calculating the person-time at risk for each subject, with start of follow-up at February 23, 2020 and end of follow-up at either the date of first positive NPS or December 26, 2020 (whichever the first).

To evaluate whether air pollution could affect the immune response of our study population we analyzed only HCWs with a positive NPS, excluding those who had performed a serological test before the NPS or after December 26, 2020. As established by regional health authorities, the serological test performed varied in time, in details:

- From February 24 to July 31, 2020, SARS-CoV-2 serology was performed with LIAISON SARS-CoV-2 S1/S2 IgG test on LIAISON XL (DiaSorin, Saluggia, Italy), a chemiluminescent immunoassay that detects quantitative anti-S1 and anti-S2 specific IgG antibodies against SARS-CoV-2 in human serum;

- From August 1 to December 26, 2020, SARS-CoV-2 serology was performed measuring anti-SARS-CoV-2 nucleocapsid antibodies (total Ig) using Elecsys anti-SARS-CoV-2 on Roche Cobas e801 (Roche Diagnostics, Monza Italy), a test based on Electro-ChemiLuminescent Immuno Assay (ECLIA).

Since the two assays were not comparable, we stratified our analyses accordingly (for brevity, we will refer to the two assays with the names of the respective manufacturers, i.e., DiaSorin and Roche).

2.3. Exposure assessment

Air pollution data were obtained from the monitoring stations of the environmental protection agency of the Lombardy region (ARPA Lombardia) for the years 2019–2020 (https://dati.lombardia.it/). Data on temperature and humidity were collected as well. Each subject was assigned the daily mean levels of particulate matter with diameter $\leq$10 μm (PM10), nitrogen dioxide (NO_2), ozone (O_3), temperature, and humidity measured by the monitoring station nearest to his/her residential address. Apparent temperature was calculated as summary meteorological variable (Analitis et al., 2008). We used the web tool GPS Visualizer (https://www.gpsvisualizer.com/) to translate addresses into spatial coordinates and linked air pollution and meteorological data using QGIS (QGIS.org, 2021. QGIS Geographic Information System. QGIS Association. https://www.qgis.org). Within each person-day, short-term exposure was calculated as moving averages of air pollution from lag 0–1 (average of the day at risk and of the day before) to lag 0–30 (average of the day at risk and of each preceding day up to 30 days before). Long-term exposure was modeled as average concentration of the pollutants of interest for the year 2019.

2.4. Statistical analysis

We calculated summary statistics (absolute number, proportion, median, interquartile range) of the main variables of interest and verified whether their distribution differed across categories of NPS (Wilcoxon-Mann-Whitney *U* test for continuous variables and chi-squared test for categorical variables).

To assess the association between short-term air pollution exposure and probability of having a positive NPS, we applied Poisson models calculating incidence rates and their ratios (IRR). In long-term analyses, we implemented Poisson models with robust estimator of the standard error. All models were adjusted for age (continuous), sex, body mass index (BMI, continuous), smoking habit (three categories), occupation (seven categories) and calendar month (11 categories). Results are reported as IRR, with corresponding 95% confidence intervals (95%CI) for an increase in the pollutant concentration of 10 $\mu g/m^3$ in short-term analyses and of 1 $\mu g/m^3$ in long-term analyses.

When focusing on antibody titer among NPS-positive subjects, we applied Poisson models adjusted for age, sex, BMI, smoking habit, occupation, and time between NPS and serological assay. In long-term analyses, standard errors were calculated using robust estimator. Results are reported as percent change in IgG levels and corresponding 95%CI for an increase in the pollutant concentration of 10 $\mu g/m^3$ in short-term analyses and of 1 $\mu g/m^3$ in long-term analyses.

For both outcomes of interest (i.e., NPS positivity and antibody titer), we also ran additional models mutually adjusting for PM10, NO_2 and O_3. Finally, we performed sensitivity analyses further adjusting for apparent temperature and stratifying the analyses according to BMI values (<25 vs. $\geq$ 25 kg/m^2).

3. Results

3.1. Characteristics of the study population

Our study population included 3712 HCWs with available information on residential address and meeting the inclusion criteria (Table 1). Median age was 46 years and females represented almost 71% of the entire population. Fifty-nine percent of HCWs had a normal weight and more than half of them were nonsmokers. Nurses were the most represented occupation (32.2%), followed by physicians (23.2%), and health technicians (15.5%). 635 subjects (17%) had at least one positive swab. Characteristics were similar among swab-positive and negative HCWs, except for smoking status where swab-negative HCWs had a higher proportion of current smokers (24%). Among the 635 swab-positive subjects, we excluded 35 with missing information on the result of the serological test and 263 since serology was performed either before NPS or after the end of the follow-up. Of the 337 included serological tests, 86 (25.5%) were performed with DiaSorin and 251 (74.5%) with Roche kits.

3.2. Air pollution data

Daily mean concentrations of PM10, NO_2 and O_3 in 2020 highlighting also national lockdown and regional mobility restriction periods are depicted in Supplementary Fig. 1: they ranged (min-max) from 5.8 to 89.4, 7.9 to 66.9, and 4.6–117.2 $\mu g/m^3$, respectively. Corresponding annual means in 2019 (used for long-term exposure) were 27.4, 30.2, and 50.7 $\mu g/m^3$.

3.3. Air pollution and risk of SARS-CoV-2 infection

When we examined short-term exposure and probability of SARS-CoV-2 infection (Fig. 1 and Supplementary Table 1), we found a

Table 1
Characteristics of the study population, Lombardy, Italy, February–December 2020.

N (%)/Median (*Interquartile Range*)	Total	Swab+	Swab-
	3712	635 (*17.1*)	3077 (*82.9*)
Age	46 (*33–54*)	45 (*33–54*) p = 0.551[a]	46 (*33–54*)
Sex			
Males	1079 (*29.1*)	202 (*32.8*)	877 (*28.5*)
Females	2633 (*70.9*)	433 (*68.2*) p = 0.095[b]	2200 (*71.5*)
Body Mass Index (BMI)	23.29 (*20.8–26.2*)	23.49 (*21.0–26.8*) p = 0.096[a]	23.2 (*20.8–26.1*)
$<$ 18 kg/m^2	117 (*3.2*)	20 (*3.1*)	97 (*3.2*)
$\geq$ 18 kg/m^2 and $<$ 25 kg/m^2	2197 (*59.2*)	362 (*57*)	1835 (*59.6*)
$\geq$ 25 kg/m^2 and $<$ 30 kg/m^2	857 (*23.1*)	142 (*22.4*)	715 (*23.2*)
$\geq$ 30 kg/m^2	313 (*8.43*)	66 (*10.4*)	247 (*8*)
Missing	228 (*6.14*)	45 (*7.1*) p = 0.236[b]	183 (*6*)
Smoke			
No	2022 (*54.4*)	389 (*61.3*)	1633 (*53.1*)
Ex	636 (*17.1*)	110 (*17.3*)	526 (*17.1*)
Yes	824 (*22.2*)	92 (*14.5*)	732 (*23.8*)
Missing	230 (*6.2*)	44 (*6.9*) p $<$ 0.001[b]	186 (*6.0*)
Occupation			
Physicians	861 (*23.2*)	138 (*21.7*)	723 (*23.5*)
Residents	237 (*6.4*)	35 (*5.5*)	202 (*6.6*)
Nurses	1194 (*32.2*)	217 (*34.2*)	977 (*31.8*)
Midwives	103 (*2.8*)	18 (*2.8*)	85 (*2.8*)
Healthcare assistants	295 (*7.9*)	58 (*9.1*)	237 (*7.7*)
Health Technicians	575 (*15.5*)	89 (*14*)	486 (*15.8*)
Clerical workers, technicians	419 (*11.2*)	79 (*12.5*)	340 (*11.1*)
Missing	28 (*0.8*)	1 (*0.2*) p = 0.438[b]	27 (*0.9*)

[a] Wilcoxon rank-sum (Mann–Whitney) test.
[b] Chi-squared test.

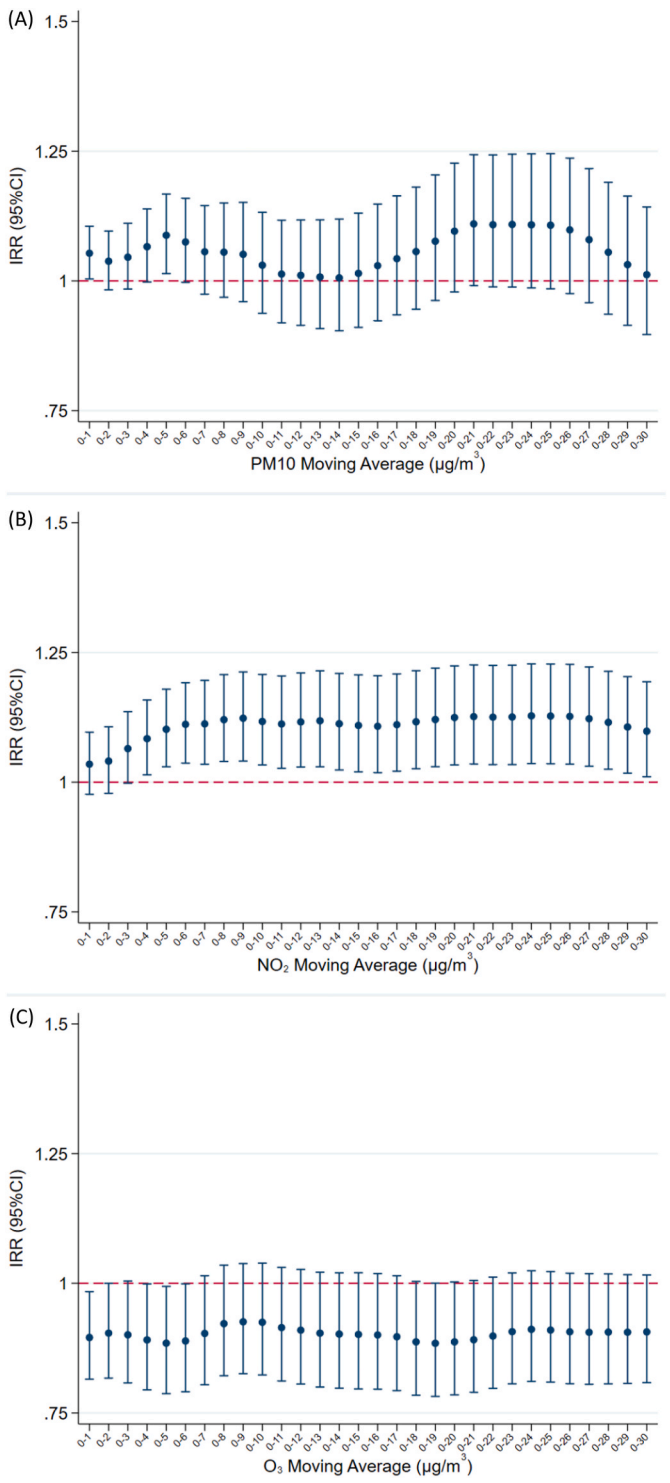

Fig. 1. Short-term exposure to PM10 (A), NO_2 (B), O_3 (C) and probability (IRR) of SARS-CoV-2 infection.

positive association with NO_2, with IRRs increasing from lag 0–4 (1.08, 95%CI: 1.01; 1.16) up to lag 0–8 (1.12, 95%CI: 1.04; 1.21) and then remaining somewhat stable up to lag 0–30 (Fig. 1B). No clear trend was observed for PM10 (Fig. 1A) while models on O_3 returned IRRs mostly below one (Fig. 1C). In the multipollutant models, the pattern of the associations remained substantially unchanged for PM10 and NO_2 (Supplementary Figs. 2 and 3 and Supplementary Tables 3 and 4), while IRRs for O_3 became closer to the null value (Supplementary Fig. 4 and Supplementary Table 5).

When evaluating long-term exposures to single pollutants we found similar findings, with an increased risk of infection observed only for NO_2 (IRR: 1.02, 95%CI: 1.00; 1.03). Multipollutant models did not alter the findings for NO_2 and O_3, whereas a slightly reduced risk was observed for PM10 in the tri-pollutant model (Table 2).

3.4. Air pollution and immune response in swab-positive subjects

A further step was to explore the association between air pollutants and immune response among swab-positive subjects. Statistical models run on the 86 HCWs with serology from DiaSorin were unstable due to the small sample size. We thus focused on the 251 HCWs with serology assessed through the Roche kit. We did not observe an evident association for PM10, NO_2 and O_3 (Fig. 2 and Supplementary Table 2).

Multipollutant models confirmed the absence of a clear trend for PM10 (Supplementary Fig. 5 and Supplementary Table 6) and O_3 (Supplementary Fig. 7 and Supplementary Table 8). Instead, a positive association between antibody titer and NO_2 was detected, especially in the tri-pollutant model and moving towards the monthly average of exposure (Supplementary Fig. 6 and Supplementary Table 7).

When we assessed long-term exposure to single pollutants, we found no effect on antibody titer for O_3 exposure while we observed an increasing percent change for PM10 (+7.4%, 95%CI: 2.5; 12.5) and NO_2 (+2.4%, 95%CI: 1.1; 3.6%). However, only the latter association was confirmed in models considering all pollutants (Table 3).

Further adjustment for apparent temperature and stratification for BMI did not substantially change our estimates (results not shown).

4. Discussion

In a large sample of HCWs working during the first year of the COVID-19 pandemic, both short- and long-term exposures to NO_2 were associated with an increased risk of SARS-CoV-2 infection. IRRs were significantly increased from lag 0–4 with estimates ranging from 1.08 up to 1.13 when considering the average concentration of the month preceding the NPS. Even for long-term exposure we observed a 3% increase in the probability of infection for each increase of 1 $\mu g/m^3$ of NO_2. No clear associations emerged for PM10 and O_3.

When we examined the antibody titer (IgG) among positive subjects, both short- and long-term exposures to NO_2 showed a consistent positive association in single as well as multi-pollutant models. No clear trends emerged for PM10 and O_3.

Few studies used individual data to examine the association between air pollution and risk of infection evaluated as positivity to NPS (Nobile et al., 2022; Sheridan et al., 2022; Stafoggia et al., 2023; Veronesi et al., 2022) or serological tests (Kogevinas et al., 2021). All studies looked at long-term exposure only.

The Spanish study involved a large cohort of subjects in Catalonia and did not highlight associations between the considered pollutants (PM2.5, NO_2 and O_3) and the risk of infection (positive serological test) between June and November 2020 (Kogevinas et al., 2021). Among the infected subjects, NO_2 and PM2.5 exposures were positively associated to IgG levels.

The more recent UK Biobank study (Sheridan et al., 2022) evaluated

Table 2
Long-term exposure to PM10, NO_2, O_3 and probability [IRR (95%CI)] of SARS-CoV-2 infection, per increment of 1 $\mu g/m^3$, Lombardy, Italy, February–December 2020.

	PM10	NO_2	O_3
Single pollutant	1.00 (0.95; 1.05)	1.02 (1.00; 1.03)	0.97 (0.94; 1.01)
Adjusted for PM10	–	1.03 (1.01; 1.05)	0.97 (0.93; 1.02)
Adjusted for NO_2	0.94 (0.89; 1.00)	–	1.00 (0.95; 1.04)
Adjusted for O_3	0.98 (0.93; 1.03)	1.02 (1.00; 1.04)	–
Tri-pollutant	0.94 (0.88; 1.00)	1.03 (1.01; 1.05)	0.98 (0.94; 1.03)

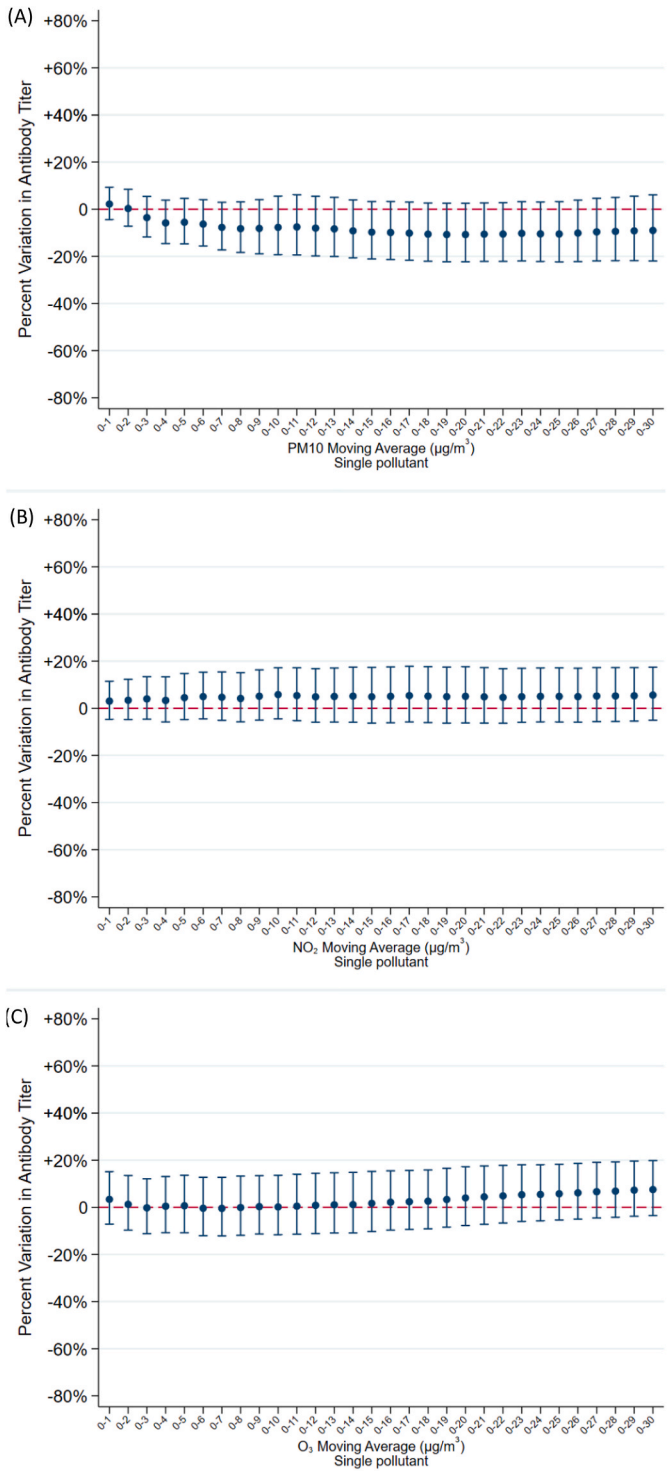

Fig. 2. Short-term exposure to PM10 (A), NO_2 (B), O_3 (C) and percent variation in antibody titer.

the associations between long-term air pollution and multiple COVID-19 outcomes (nasal swab positive tests, hospitalization, and mortality) between March and December 2020. In this large prospective cohort study, a positive association between PM2.5, NO_2 and COVID-19 positive test was found after adjusting for relevant confounders (demographics and lifestyle factors).

Three other studies examined the association of air pollution with COVID-19 infection in Italy. A positive association between annual averages of PM2.5, PM10, NO_2, NO and incidence of SARS-CoV-2 infection

Table 3
Long-term exposure to PM10, NO_2, O_3 and percent variation in antibody titer [PV (95%CI)], per increment of 1 μg/m^3, Lombardy, Italy, February–December 2020.

	PM10	NO_2	O_3
Single pollutant	7.4 (2.5; 12.5)	2.4 (1.1; 3.6)	−2.9 (−6.0; 0.4)
Adjusted for PM10	–	1.9 (0.2; 3.6)	−0.9 (−4.7; 3.0)
Adjusted for NO_2	2.3 (−3.6; 8.8)	–	−0.5 (−4.1; 3.2)
Adjusted for O_3	6.9 (1.4; 12.8)	2.3 (0.9; 3.8)	–
Tri-pollutant	2.2 (−4.0; 8.9)	1.9 (0.2; 3.7)	−0.2 (−3.9; 3.6)

(NPS positive test) was found in a prospective study of residents in the city of Varese (Northern Italy) (Veronesi et al., 2022), whereas the COVID-19 positive subject rate was not associated with long-term PM2.5 and NO_2 exposure in a large cohort of residents in Rome, Central Italy (Nobile et al., 2022). The latter study lacked data on individual lifestyle characteristics although demographics, socioeconomic and neighborhood characteristics had been accounted for. In the most recent investigation considering the entire Italian territory and retrieving data on swab-positive subjects based on a national surveillance system (Stafoggia et al., 2023), positive associations were found between long-term exposure to PM10, PM2.5 and NO_2 and SARS-CoV-2 infection, especially among the elderly. Results most robust to sensitivity analyses were those regarding NO_2.

Our findings are partially consistent with those described above indicating a positive association between NO_2 exposure and an increased risk of COVID-19 infection measured as NPS positive tests. Moreover, the increase in the antibody response with increasing exposure to NO_2 that we observed is consistent with the results of the study by Kogevinas and colleagues (Kogevinas et al., 2021), which showed a similar trend among subjects with a positive serological test. In addition, also the most recent investigation on the Italian territory (Stafoggia et al., 2023) seems to confirm the stronger role of NO_2 in influencing the risk of infection.

Although toxicological evidence suggests that exposure to particulate matter may contribute to the severity of COVID-19 disease by altering the immune response and host defenses, and in particular by suppressing the adaptive immune response to virus infection, some studies have reported an increase in immunoglobulins in PM-exposed subjects (Leonardi et al., 2001). In a group of school-age children, enrolled in the context of the European multicenter study CESAR (Central European Study of Air Quality and Respiratory Diseases), an increase in some inflammatory and total IgG indices with increasing PM2.5 concentrations was observed (Leonardi et al., 2001). A similar result with increased IgG, IgM and IgE has been described in traffic police officers exposed to high concentrations of PM2.5 in China (Zhao et al., 2013): the authors hypothesize that long-term exposure to particulate matter induces chronic inflammation of the respiratory tract with an increase in the humoral immune response.

Studies examining the specific role of NO_2 are very scarce. However, NO_2 can easily reach alveolar macrophages and affect their ability to start an antiviral immune response (Saghazadeh and Rezaei, 2022). Indeed Feng and colleagues, evaluating the impact of short-term air pollution exposure, showed a downregulation in genes involved in antiviral defense processes resulting in suppression of virus clearance in a subset of COVID-19 cases highly-exposed to NO_2 compared to low exposed ones (Feng et al., 2024). In addition, NO_2 has been shown to increase susceptibility to other viral respiratory infections, such as influenza and respiratory syncytial virus (Becker and Soukup, 1999; Goings et al., 1989). Finally, although limited by its ecological design, a study assessing COVID-19 fatality in 66 administrative European regions observed how the five regions with the highest NO_2 concentrations were also those with the worst fatality rates (Ogen, 2020).

Results on the mechanisms underlying the association between different air pollutants and respiratory infections are thus not fully

consistent, as also documented by a recent review reporting findings from *in vitro* and animal model studies, which describes how different immune response mechanisms can be fostered by different air pollutants (Burbank, 2023). Further studies evaluating the effect of air pollution on the immune response to specific infections such as SARS-CoV-2 are thus warranted.

Our study has several strengths. First, we could rely on a well-characterized cohort of HCWs, which allowed us to thoroughly adjust for several individual confounders. We decided to stop the follow-up before the starting of vaccination campaign. This decision surely restricted our population size but, on the other hand, insured us a greater robustness of our outcomes: the vaccine could not influence the probability of testing positive or the value of antibody titer. Finally, all components of our population share similar probabilities of both being infected and undergoing test swabs. For these reasons, analyzing this population reduces some biases related to different characteristics between swab-positive and negative subjects: in the general population, especially in the period that we analyzed, people underwent swab tests because of symptoms or contact with a case. Most HCWs, and our population for sure, also underwent testing for mandatory periodical screening: we were thus able to include also asymptomatic subjects and avoid selection bias, which is often present in studies with aggregate data (Villeneuve and Goldberg, 2020).

Our study has also limitations. First, our results might be hampered by the small sample size of our study population, especially in the sub-analysis on antibody titer. Second, our findings rely on a peculiar workforce and may thus not directly apply to the general population. Third, many of our study subjects live in Milan and share similar air pollution exposure levels (if swabbed in similar time periods). Fourth, some subjects might live in rural places, where the "nearest" monitoring station is placed far from their residential address, thus potentially entailing misclassification of the attributed exposure.

5. Conclusion

In conclusion, in a cohort of HCWs with available individual data, we found a positive association between short- and long-term exposure to NO_2 and risk of infection for SARS-CoV-2. The same exposures were also associated to a higher antibody titer among positive subjects. Notwithstanding its peculiarities, our study contributes to increase the body of evidence suggesting an active role of air pollution exposure on SARS-CoV-2 infection and confirms the importance of implementing pollution reduction policies to improve public health (Coccia, 2023).

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Ethics approval

The study was approved by the institutional review board (368_2020bis) of our hospital (Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.116755.

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